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# *In vitro* Organogenesis Secondary Metabolite Production and Heavy Metal Analysis in *Swertia chirayita*

**Research Article**

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**Abstract:** An efficient protocol of plant regeneration through direct and indirect organogenesis in *Swertia chirayita* was developed. Explants cultured on Murashige and Skoog medium supplemented with 2,4-D (0.5 mg $L^{-1}$) with combination of Kinetin (0.5 mg $L^{-1}$) showed the highest frequency (84%) of callusing and 1.0mg $L^{-1}$ 6-benzyladenine (BA) in combination with (100 mg $L^{-1}$) Adenine sulphate (Ads) + (0.1 mg $L^{-1}$) Indole acetic acid (IAA) was excellent for maximum adventitious shoot (12.69 ± 1.30) formation in four week of culture. A maximum number of (7.14 ± 0.99) shoots were developed per leaf explants through direct organogenesis. The highest frequency of rooting (11.46 ± 1.56) was observed on MS medium augmented with IAA (1.0 mg $L^{-1}$). Well-rooted shoots transferred to plastic pots containing a soilrite: sand mix and then moved to the greenhouse for further growth and development. Four major secondary metabolites were analyzed and quantified using high performance liquid chromatography. Amount of secondary metabolites was found significantly higher, in *in vitro* plantlets compared to *in vivo* plantlets and callus raised from *S. chirayita*. Higher heavy metal accumulation in *in vitro* as compared to *in vivo* plantlets correlates higher secondary metabolite production supporting that they play regulatory role in influencing the plant secondary metabolism.

**Keywords:** *Organogenesis • High Performance Liquid Chromatography • Swertia chirayita • Secondary metabolites*



## Abbreviations

| | |
|---|---|
| Ads | - Adenine sulphate |
| BA | - 6-Benzyladenine |
| HPLC | - High Performance Liquid Chromatography |
| Kn | - Kinetin |
| NAA | - Naphthalene acetic acid |
| PGRs | - Plant growth regulators |
| 2,4-D | - 2,4-Dichlorophenoxyacetic acid |
| ICP – OES | - Inductively coupled plasma optical emission spectroscopy |
| MS | - Murashige and Skoog |

## 1. Introduction

*Swertia chirayita* (Roxb. ex Fleming. H. Karst.) is an indigenous medicinal herb, belonging to the family Gentianaceae. This medicinal plant is native to temperate Himalayas and found at an altitude of 1200 – 3500m. *S. chirayita* is used as herbal medicine for various health ailments including liver disorders, malaria, gastrointestinal infections and diabetes and it has been used in Unani medicine [1]. Extracts of *S. chirayit*a have been shown to possess antioxidative, antihepatotoxic and hypoglycemic, anti-inflammatory, antimalarial, anticarcinogenic, and antimicrobial activities [2-4]. This herb being so medicinally important needs conservation and propagation. Seed germination rate of *S. chirayita*

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is also very poor [5]. Only a few scattered reports in the literature suggest germination studies and nursery practices of *S. chirayita* [6]. Direct organogenesis from explants, escaping the callus induction phase, is desirable especially in modern breeding where increasing rapidity and reducing costs of regeneration are essential. Direct organogenesis in *S. chirayita* was investigated by different explants such as nodal explants [1], shoot tip [7], leaf explants [8] and immature seed cultures [9]. Efficient regeneration protocols from shoot tip of field grown plants and high frequency somatic embryogenesis and regeneration from synthetic seed has previously published [10,11]. Callus induction from different explants of *Swertia angustifolia* and indirect regeneration from calli in *Swertia mussotti* was reported respectively [12,13]. Traditional uses, demand from pharamaceutical industries and research activities adversely affect the natural population of this valuable medicinal herb. Previous reports documented the presence of flavonoids, xanthones, terpenoids, iridoid and secoiridoid glycosides, that are responsible for therapeutic properties in *S. chirayita* [14]. Mangiferin has been reported to possess various biological activities like antitumour, antiviral, antioxidant, antidiabetic and immunomodulatory activity [15-17]. Swertiamarin, Amarogentin and Sweroside compounds also possesses various biological activities such as chemopreventive, antibacterial, anticholinergic and antihepatitis activity and served as important chemotaxonomic markers [18-20]. Hence conservation and multiplication of this valuable medicinal herb *S. chirayita* is highly demanding. Therefore, the main objective of this study is to obtain high frequency regeneration of *S. chirayita* to extract the high value active secondary metabolites. The present study examined the role of different plant growth regulators (PGRs) on *in vitro* callus induction and regeneration and a comparative study of its active metabolites through high performance liquid chromatography. To our knowledge, the present study would be the first report on in direct organogenesis of *S. chirayita* through callus culture *via* leaf explants collected from wild grown plantlets and qualitative and quantitative analysis of secondary metabolites from *in vivo*, *in vitro* cultures and callus tissue of *S. chirayita*.

# 2. Experimental Procedures

## 2.1 Plant Material and culture conditions

*Swertia chirayita* (Roxb. ex Fleming) H. Karst plants were collected from their natural habitat of Darjeeling, West Bengal during the month of November. *In vivo* grown leaves harvested from 6 to 8 month old plants were used as explant to study direct and indirect organogenetic potential. Excised leaves were surface sterilized by soaking in 0.2% Bavastin (fungicide) solution followed by Tween 20, (5-6drops/100ml) solution for 20 min. Finally, the leaves were surface sterilized with 0.1% $HgCl_2$ w/v for 8 min and thoroughly rinsed 4-5 times with sterilized distilled water. In a laminar air flow cabinet sterilized explants were cut into a small discs and inoculated in MS medium [21] supplemented with 3% sucrose (w/v), 0.8% agar and various PGRs. Before autoclaving the medium for 20 min at 121ºC, the pH was adjusted to 5.8. Cultures were maintained in a growth chamber with a 16 h/8 h light/dark photoperiod at 22 ± 2ºC. Light was supplied at intensity of 80 µmol $m^{-2}s^{-1}$ by cool-white fluorescent lamps.

## 2.2 Callus induction and proliferation

For induction and establishment of callus, surface sterilized leaf explants were excised 1 cm in length, and cultured in MS medium supplemented with different concentrations and combinations of PGRs. The medium was further augmented with (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg $L^{-1}$) 2,4-Dichlorophenoxy acetic acid(2,4-D) individually and with combination of (1.0 mg $L^{-1}$) 6- Benzyladenine (BA), (0.5 mg $L^{-1}$) Kinetin (Kn) and (1.0 mg $L^{-1}$) Naphthalene acetic acid (NAA). All the cultures were incubated at 22 ± 2°C. Frequency (%) of explants that initiated callus and multiplication rate of callus was recorded after 4 weeks. Subcultures were performed at an interval of 10 days.

## 2.3 Shoot proliferation

For plantlet regeneration, the calli obtained from the callus induction medium were transferred to regeneration MS medium supplemented with BA (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg $L^{-1}$) alone and in combination with (0.1 mg $L^{-1}$) Kn, Adenine sulphate (Ads) (100 mg $L^{-1}$) + Indole acetic acid (IAA) (0.1 mg $L^{-1}$) and NAA (1.0 mg $L^{-1}$). A set of 25 explants per treatment was cultured, and each experiment was repeated three times. The mean number of shoots induced per callus was recorded after 6 weeks of incubation.

*In vivo* sterilized leaf explants were placed on full- strength MS medium supplemented with 3% (w/v) sucrose, 0.8% (w/v) agar (Himedia) and various concentrations of BA (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg $L^{-1}$) alone and in combination with Kn (0.1 mg $L^{-1}$), Ads (100 mg $L^{-1}$) + IAA (0.1 mg $L^{-1}$) and NAA (1.0 mg $L^{-1}$). The excised leaves were placed inside the culture tubes with their abaxial surface in contact with the culture medium. A set of 25 explants per treatment was cultured, and each experiment was repeated at least thrice. All of the cultures were incubated under a 16/8 h (light/dark)

at 22 ± 2ºC. Subcultures were done at regular intervals. The number of shoots developed per leaf explant was recorded after 4 weeks.

## 2.4 Rooting and acclimatization of *in vitro* Plantlets

For *in vitro* root development, the regenerated shoots with 3-4 compound leaves were excised from culture and transferred to full strength MS medium containing 3% (w/v) sucrose and 0.8% (w/v) agar. The medium was further supplemented with IAA (1.0, 2.0 and 3.0 mg $L^{-1}$), Indole butyric acid (IBA) (1.0, 2.0, and 3.0 mg $L^{-1}$) and NAA (1.0, 2.0 and 3.0 mg $L^{-1}$) separately. The number of roots developed per shoot was recorded after 4 weeks. Rooted plantlets were detached from *in vitro* culture, washed properly with distilled water and transferred to plastic pots containing soilrite : sand (1:1; 1:2; 1:3 v/v), and covered with transparent plastic. Plantlets were grown under a natural light environment at 24 ± 1°C (day) and 20 ± 1°C (night). After this time, the plants were transferred to greenhouse conditions (30–35°C, relative humidity 70%, light 127 µmol $m^{-2}s^{-1}$). After 1 week, the plastic covers were removed and shifted to pots progressively to allow for the acclimatization of the plants.

## 2.5 Extraction and quantification of secondary metabolites

*In vivo* and *in vitro* acclimatized plant samples and calli were dried at room temperature of 20-25ºC. The plant parts were grinded to make a fine powder. One gram of each dried samples was melted with 100 ml of methanol (to make 1%, w/v extract). This mixture was left at room temperature for overnight. The sample was then filtered the next day using Whatmann No 1 filter paper.

Analytes were separated using a Waters Acquity HPLC system (Waters Corporation, Milford, MA) consisting of waters 510 HPLC pump, an autosampler, an Acquity Tunable absorbance detector and temperature containing module containing (R) $C_{18}$ column (4.6 mm × 150 mm; 4 µm particle size).

Plant extracts were filtered through a 0.2 µm filter and 10 µl of extracts were injected in a HPLC system. The solvents optimized for gradient elution consisted of (A) acetonitrile and (B) 0.1% trifluroacetic acid in Milli Q water. A linear gradient elution program was applied as follows: 0-4 min : 10% A, 90% B; 4-12 min: 10% A, 90% B; 12-16 min: 30%, 70%B; 16-20 min: 40%A, 60%B; 20-24 min: 70%A, 30%B; 24-25 min: 70%A, 30%B; 25-30 min: 10% A, 90%. The flow rate was maintained at 1.0 ml $min^{-1}$. Injection volumes were 10 µl for standards as well as for samples. The detector was set at 254 nm and instrument operations, data acquisition and processing were performed using EmPower2 chromatographic data software (Waters Corporation, Milford, MA). The compounds peaks from samples were identified by the comparison of retention times with the corresponding retention times of standards. Quantification was performed using HPLC and the amounts of secondary metabolites were calculated using standard curves. All experiments were repeated at least three times. The results are presented as µg $mL^{-1}$ of extracts.

## 2.6 Heavy metal analysis

The dried homogenized samples (0.2 g) of *in vitro* and *in vivo* plantlets were placed separately in borosilicate glass digestion tubes. To each of the tubes, 8 ml of nitric acid ($HNO_3$) and hydrogen peroxide ($H_2O_2$) (7:1, v/v) were added and the tubes were then placed on a heating block with the temperature set to increase to 150 °C for about 2 h or until the solutions were completely digested. The resultant liquid was diluted with distilled water up to 100 ml. The mineral contents of tissues were analyzed using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES DV 2100, Perkin Elmer, USA) with the following parameters: with Perkin Auto-Sampler with the following parameters: plasma flow rate (15 L $min^{-1}$), nebulizer flow rate (0.6 L $min^{-1}$), sample flow rate (2 mL $min^{-1}$), RF power (1450 Watts), and auxilliary flow rate (0.2 L $min^{-1}$).

## 2.7 Data analysis

Each treatment consisted of five Erlenmeyer flasks, each containing five explants (n = 25). All experiments were repeated three times. Data obtained from all experiments were presented as the mean ± standard error of three replications. Statistically significant differences were determined by analysis of variance (ANOVA) and the Duncan multiple range test (DMRT) at a $P < 0.05$ level of significance.

# 3. Results and Discussion

In the present study complete regeneration was successfully achieved from *in vivo* leaf explants of *S. chirayita* through indirect (Figure 1) and direct (Figure 2) organogenesis. *In vivo* leaf explants produced callus on MS medium supplemented with 2,4-D (0.5.1.0, 1.5, 2.0, 2.5 and 3.0 mg $L^{-1}$) alone and with combination of BA (1.0 mg $L^{-1}$), Kn (0.5 mg $L^{-1}$) and NAA (1.0 mg $L^{-1}$). The type and concentration of Plant growth regulators (PGRs) influenced induction of callus per explant.

Among various concentrations of PGRs tested, least concentration of 2,4-D (0.5 mg $L^{-1}$) in combination with Kn (0.5 mg $L^{-1}$) were found to be the optimum for

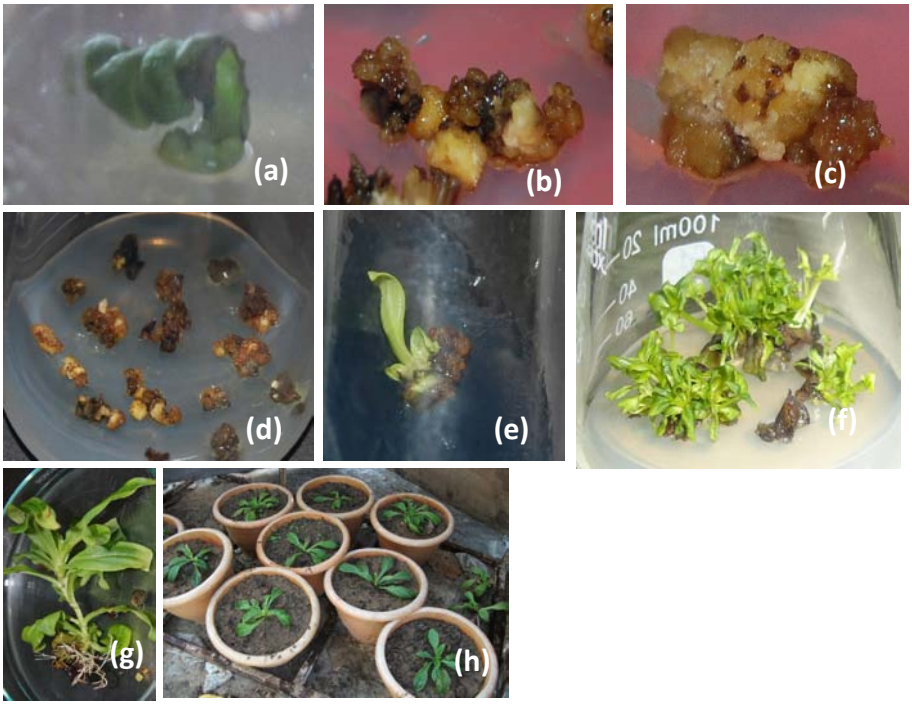


**Figure 1.** Indirect regeneration in *S. chirayita*. From leaf explants (a) swelling of leaves after one week of culture; (b-c) Callus derived from leaves on medium with 2,4-D (0.5 mg $L^{-1}$) + KN (0.5 mg $L^{-1}$) after 4 week of culture; (d) Callus multiplication on same above medium; (e) shoot bud induction from callus; (f) multiple shoot bud induction in MS+ BA (1.0 mg $L^{-1}$) with combination of Ads + IAA (100 mg $L^{-1}$+ 0.1 mg $L^{-1}$); (g) complete rooted plant of *S. chirayita* (h) acclimatized and hardened plantlets of *S. chirayita*.

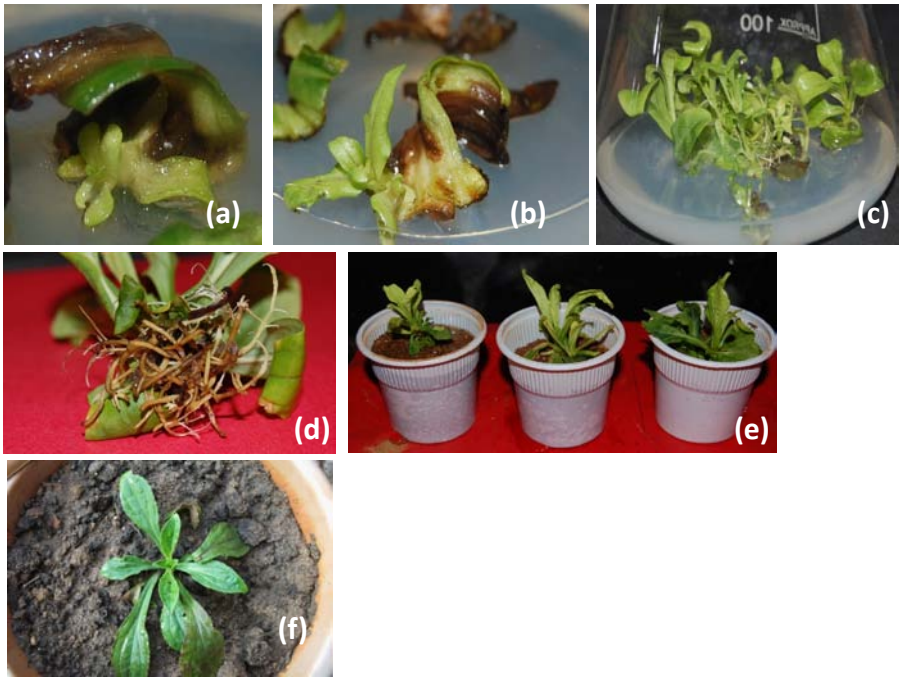


**Figure 2.** Direct regeneration in *S. chirayita* from leaf explant (a-b) Initiation of shoot buds from basal segment of leaf, (c) multiple shoot buds differentiated from leaf explant grown in MS medium with BA (1.0 mg $L^{-1}$) with combination of Ads + IAA (100 mg $L^{-1}$+ 0.1- mg $L^{-1}$); (d) complete rooted plants prior to field transfer, (e) *In vitro* acclimatized plantlets in plastic cups containing soilrite and sand in 1:2 ratio in growth room; (f) hardened plants under field condition.

callus induction. The explants showed maximum (84%) callus induction response (Figure 3) after 4 weeks of culture. MS medium containing BA (13.32 µM $L^{-1}$) with 2,4-D (0.90 µM $L^{-1}$) produced 100% callus response in *S. chirayita* from root explants after five weeks of culture [22]. Similarly, in saffron least concentration of 2,4-D (0.25 mg $L^{-1}$) in combination with and BA (1 mg $L^{-1}$) was found to be best for callus initiation [23]. In several other Gentianaceae species like *Swertia angustifolia* and *Swertia mussotti* higher concentration of 2,4-D (3.0 mg $L^{-1}$) and Kn (2.5 mg $L^{-1}$) exhibited best callusing in leaf explants [12,13].

After 6 weeks of subculture, the callus clumps were subcultured to the regeneration medium. Among various concentrations of PGRs tested, BA (1.0 mg $L^{-1}$) in combination with Ads (100 mg $L^{-1}$) and IAA (0.1 mg $L^{-1}$) were found to be the optimum in multiple shoot induction. The highest number of shoots (12.69 ± 1.30) per callus were produced (Figure 4) on the same medium. A similar PGRs combination has been reported for multiple shoot induction from callus in *S. chirayita* [24]. They reported only 10 shoots per callus clump when media was fortified with BA (8.88 µM $L^{-1}$) in combination with Ads (271.45 µM $L^{-1}$) and IAA (2.85 µM $L^{-1}$) through root derived callus after four weeks of culture.

Direct organogenesis provides an efficient regeneration and multiplication of endangered species without the intervening callus phase. Complete regeneration of *S. chirayita* was obtained through direct organogenesis using leaf explants. Leaves collected from field grown plantlets produced varied number of multiple shoots on MS medium supplemented with BA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg $L^{-1}$) alone and in combination of Kn (0.1 mg $L^{-1}$), Ads (100 mg $L^{-1}$) + IAA (0.1 mg $L^{-1}$) and NAA (1.0 mg $L^{-1}$). The type and concentration of PGRs influenced the average number of multiple shoots per explants. Among various concentrations of PGRs tested, least concentration of BA (1.0 mg $L^{-1}$) in combination with Ads (100 mg $L^{-1}$) + IAA (0.1 mg $L^{-1}$) were found to be the most effective

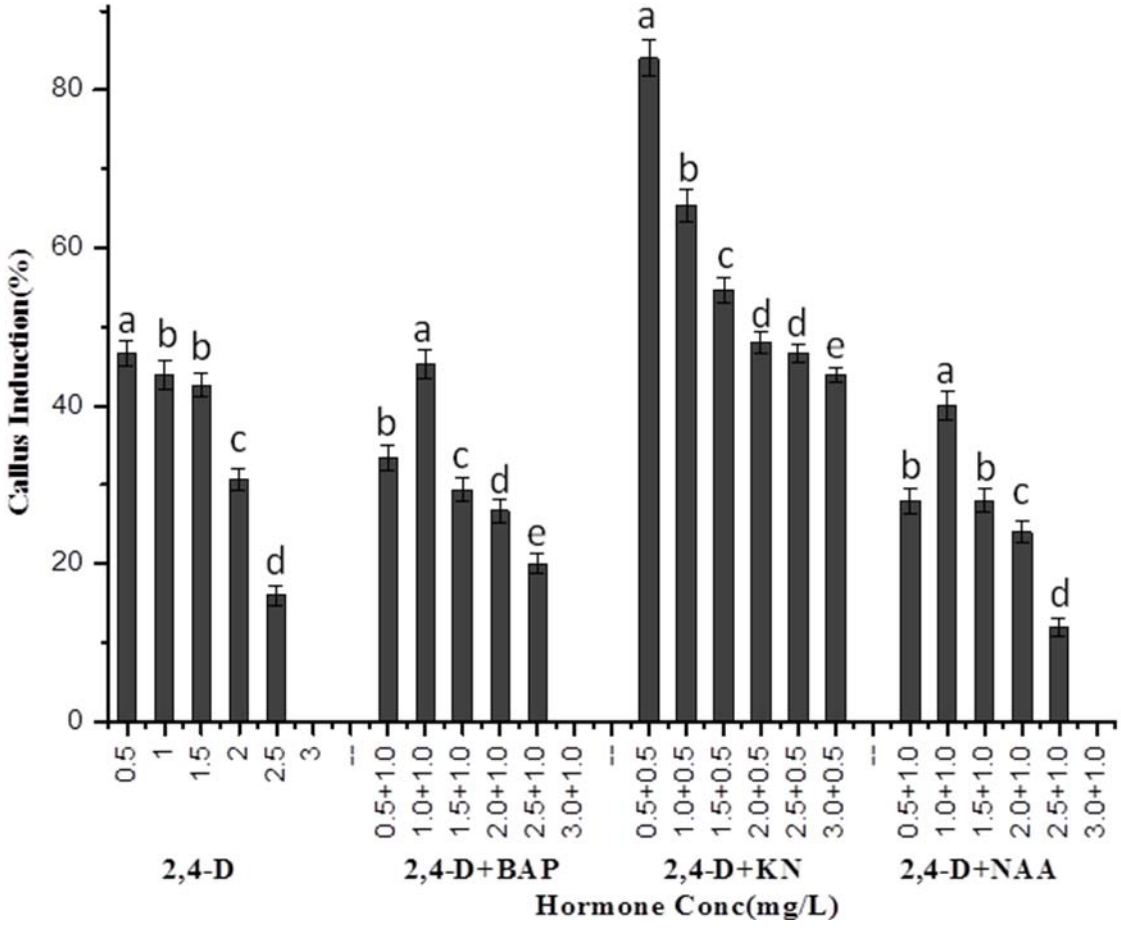


**Figure 3.** Effects of various concentrations of PGRs in MS medium with 2,4-D alone and in combination with BA, KN and NAA on callus induction of *S. chirayita*. Values represent means ± SE of three replications. In graph columns with different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

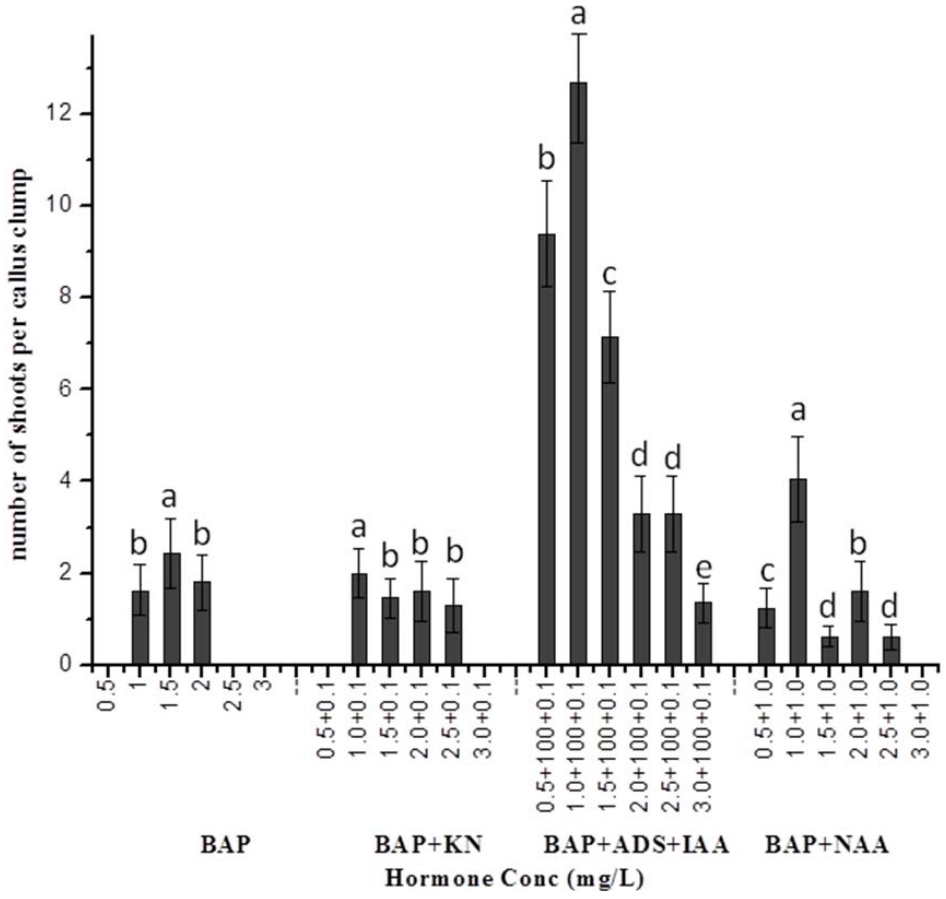


**Figure 4.** Effects of various concentrations of PGRs in MS medium with BA alone and in combination with KN, Ads + IAA and NAA on mean number of shoots from leaf derived callus of *S. chirayita*. Values represent means ± SE of three replications. In graph columns with different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

in multiple shoot induction. The maximum numbers of shoots (7.14 ± 0.99) per explant were produced (Figure 5) on MS medium containing BA (1.0 mg $L^{-1}$) in combination with Ads (100 mg $L^{-1}$) and IAA (0.1 mg $L^{-1}$) from *in vivo* leaf explants after 4 weeks of culture. More than seven shoot buds per explants were obtained from *in vitro* leaf of *S. chirayita* for the first time when the explants were placed on MS medium supplemented with 2.22 µM $L^{-1}$ N-6-benzyladenine, 11.6 µM $L^{-1}$ kinetin, and 0.5 µM $L^{-1}$ a-naphthalene acetic acid [1]. Similar number of shoots were produced from *in vivo* leaf explants of *S. chirayita* with 190-2 basal medium containing 13.32 µM $L^{-1}$ 6-BA and 0.54 µM $L^{-1}$ NAA after 4 weeks of culture [24]. Koul *et al.* [25] reported direct shoot regeneration on MS medium supplemented with (2.0 mg $L^{-1}$) BA from nodal explants for the development of axillary adventitious shoot buds after 30 days. In their study, reduced BA concentration (0.5 mg $L^{-1}$) was found to be effective for shoot proliferation. The simulative role of Ads in shoot multiplication has been emphasized from time to time in various plants [26,27]. Adenine sulphate, supplied in combination with BA and IAA, enhanced the shoot multiplication by many folds during the present investigation. In our study, addition of (100mg $L^{-1}$) Ads in MS medium was found to be optimal for multiple shoot induction and proliferation from *in vivo* leaf explants after 4 weeks. Adenine in the form of Ads can stimulate cell growth and shoot multiplication probably by acting as organic nitrogen source and/or acting as pre-cursor for natural cytokinin synthesis. A Similar strategy for using Ads as an adjuvant has also been adopted effectively for many other plant species such as *Phaseolus vulgaris*, *Holarrhena antidysenterica* Wall, *Bacopa monnieri*, *Citrus reticulata* Blanco, *Curcuma angustifolia Roxb*. [28-32].

Proliferated shoots when excised and cultured on full-strength MS medium containing different concentrations of IAA, IBA and NAA showed varied results for rooting. IAA was found to be more effective for root induction than IBA and NAA. The rooting response was 80% and the highest number of

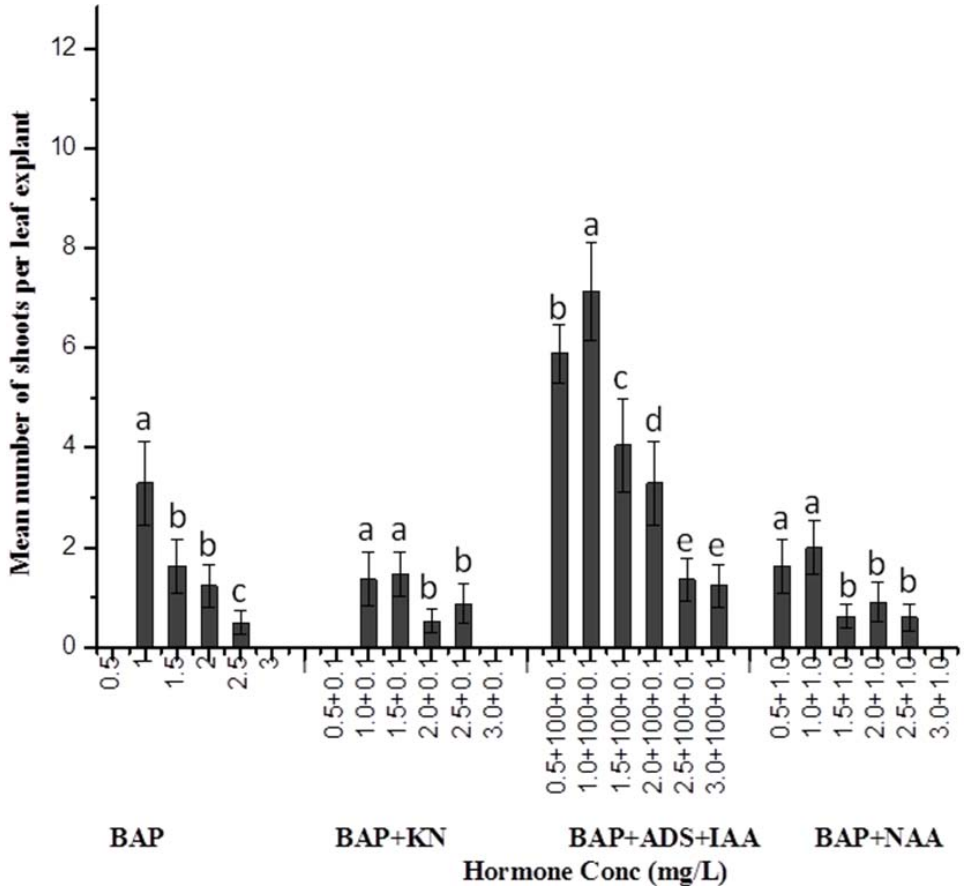


**Figure 5.** Effects of various concentrations of PGRs in MS medium with BA alone and in combination with KN, Ads + IAA and NAA on mean number of shoots per explants of *S. chirayita* from leaf. Values represent means ± SE of three replications. In graph columns with different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

roots (11.46 ± 1.56) per shoot was produced on full strength MS medium containing IAA (1.0 mg $L^{-1}$) within 4 weeks of time (Figure 6). An increased frequency of root formation and number of roots per shoot were observed in *Vigna radiata* when the medium was supplemented with IBA (4.90 µM $L^{-1}$) [33]. The combination of IBA (2.0 mg $L^{-1}$) with IAA (0.1 mg $L^{-1}$) on MS medium induced higher frequency (90%) of rooting in *Citrus reticulata* [31]. In both the studies, higher concentration of IBA induced more rooting but in our concerned study lower concentration of IAA was more efficient with respect to IBA. MS medium supplemented with IAA ranging between 1-5 mg $L^{-1}$ showed 60–65% rooting in *S. chirayita* in 8 weeks of time duration [34]. But in our present study 80% rooting were observed when IAA (1.0 mg $L^{-1}$) was used. After 4 weeks of root induction, the plantlets with fully expanded leaflets with a height of 4-5 cm were washed to remove all adhering culture medium and then successfully hardened in the culture room (22 ± 2ºC), with sterilized planting substrates soilrite: sand (1:1; 1:2; 1:3 v/v) for 3-4 weeks. Of the different types of planting substrates examined, percentage survival of the plantlets was highest (80%) in soilrite and sand in 1:2 ratio. Roots developed by excised shoots of *S. chirayita* were viable, and plantlets were successfully acclimatized to *ex-vitro* conditions.

For qualitative and quantitative analysis of plant secondary metabolites, four reference compounds procured from chromadex™ viz. swertiamarin, mangiferin, amarogentin and sweroside were used in the experiment. In the previous studies only qualitative analysis of xanthone and secoiridoid glycosides were reported from *S. chirayita* [35]. In *Centaurium pulchellum*, the secoiridoids and xanthones were isolated and quantified in the shoots and roots [36]. In their study, amount of secondary metabolites in *in vitro* tissue was higher when compared to the *in vivo* plantlets. Jankovic *et al.* [37] also reported higher amounts of xanthone compounds in *in vitro* raised plantlets in comparison to naturally growing plantlets in *Centaurium erythraea*. A similar results

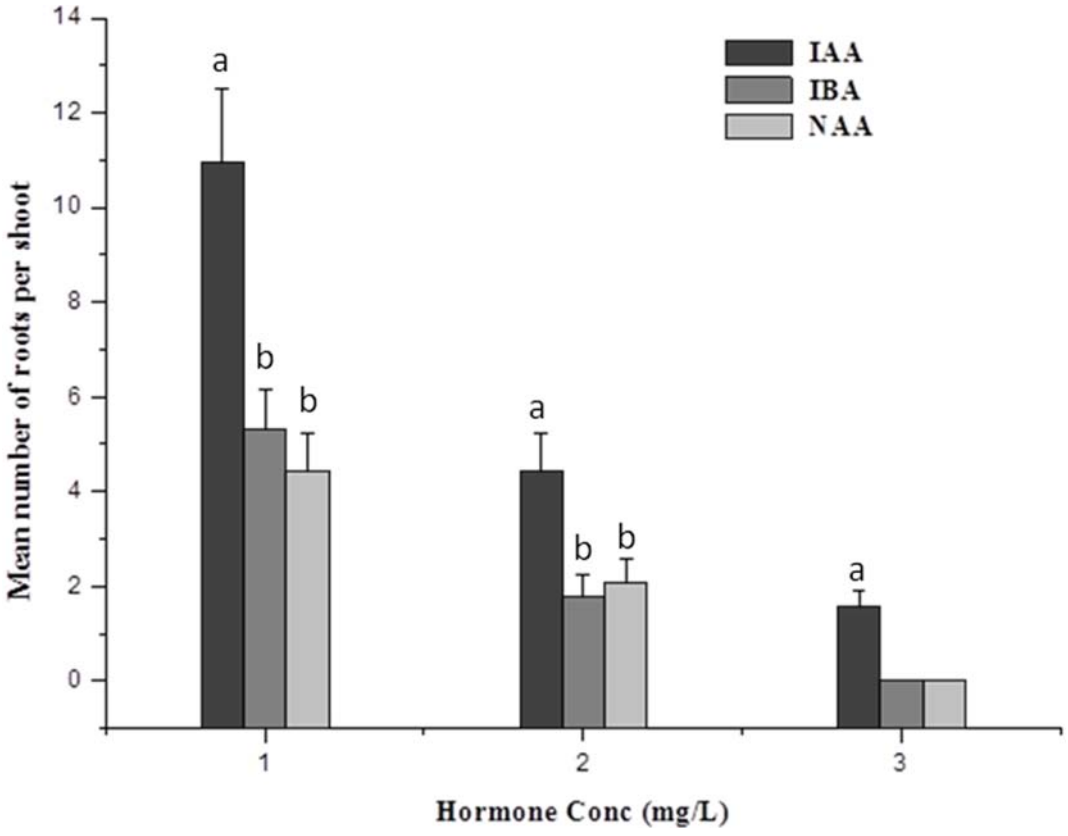


**Figure 6.** Effects of auxins on root induction from regenerated *S. chirayita* shoots. Values represent mean ± SE of three replications. In graph columns with different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

were obtained in the present study also where amount of secondary metabolites were higher in *in vitro* tissues compared to the *in vivo* plantlets of *S. chirayita* (Figure 7). Production of secondary metabolites in several Gentianaceae species like *Swertia japonica* [38], and *Gentiana lutea* [39] has been reported previously. In our present study, Figure 8 shows the total swertiamarin, amarogentin, mangiferin and sweroside contents in regenerated plantlets, *in vivo* plantlets and callus extract of *S. chirayita*. The highest swertiamarin (11.08 ± 0.63) µg $mL^{-1}$ and mangiferin (14.60 ± 0.33) µg $mL^{-1}$ content were observed in regenerated plantlets whereas highest amarogentin (29.09 ± 0.81) µg $mL^{-1}$ and sweroside (72.20 ± 1.08) µg $mL^{-1}$ content were found in *in vivo* plantlets but swertiamarin were absent. In contrast to this, low amount of swertiamarin (8.23 ± 0.17) µg $mL^{-1}$, amarogentin (2.65 ± 0.08) µg $mL^{-1}$ and sweroside (10.62 ± 0.33) were observed in callus extract whereas mangiferin was absent. Except sweroside, *in vitro* regenerated plantlets showed the highest amount of secondary metabolites compared to *in vivo* plantlets and callus extract.

ICP-OES is a powerful tool for the determination of heavy metals in a variety of different sample matrices [40]. Metal ions also influenced secondary metabolite production [41]. The concentration levels of metals found in both the samples are summarized in Table 1. Highest Ca, Fe and Mn were found in *in vitro* plantlets compared to *in vivo* plantlets of *S. chirayita*. The rest of the metals are found to be similar. Effective accumulation of metals like Cr, Fe, Mn also produced an increase of secondary metabolite like *Brassica juncea* [42]. Increase in $Ca^{2+}$ concentration followed by the activation of $Ca^{2+}$-cascade is an essential cellular event for the enhancement of the biosynthesis of natural products in plant cells [43]. Therefore we can conclude that due to presence of highest amount of Ca, Fe and Mn, amount of secondary metabolite found higher in *in vitro* plantlets compared to *in vivo* plantlets of *S. chirayita*. More study in relation to identification and characterization of the other secondary metabolites being secreted by the *in vitro* cultures of *S. chirayita* is still awaited.

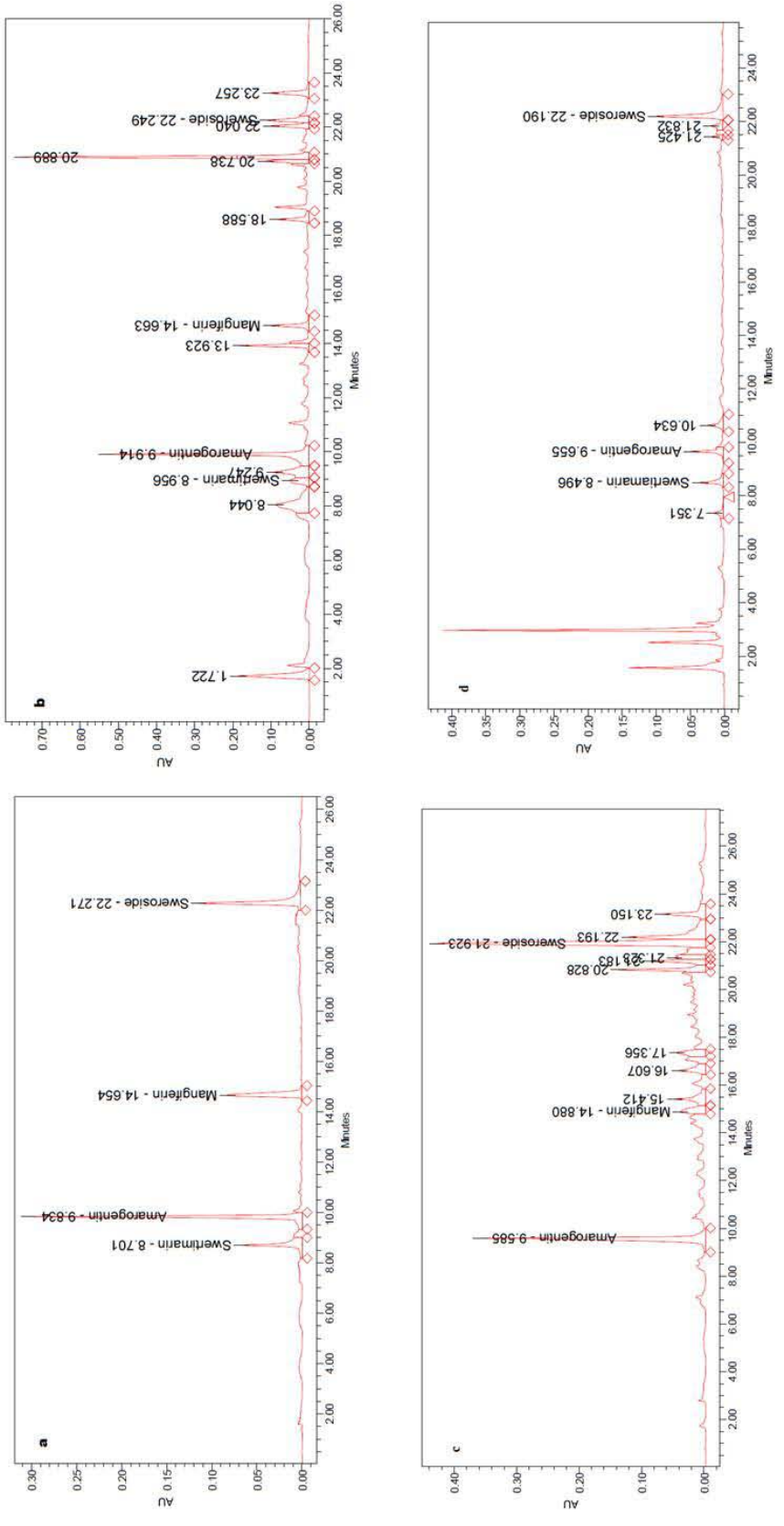


**Figure 7.** HPLC profiles of (a) mixture of standards *i.e.*, swertiamarin, amarogentin, mangiferin and sweroside; (b) methanol extract of regenerated *in vitro* plantlets; (c) *in vivo* plantlets; (d) and callus extract.

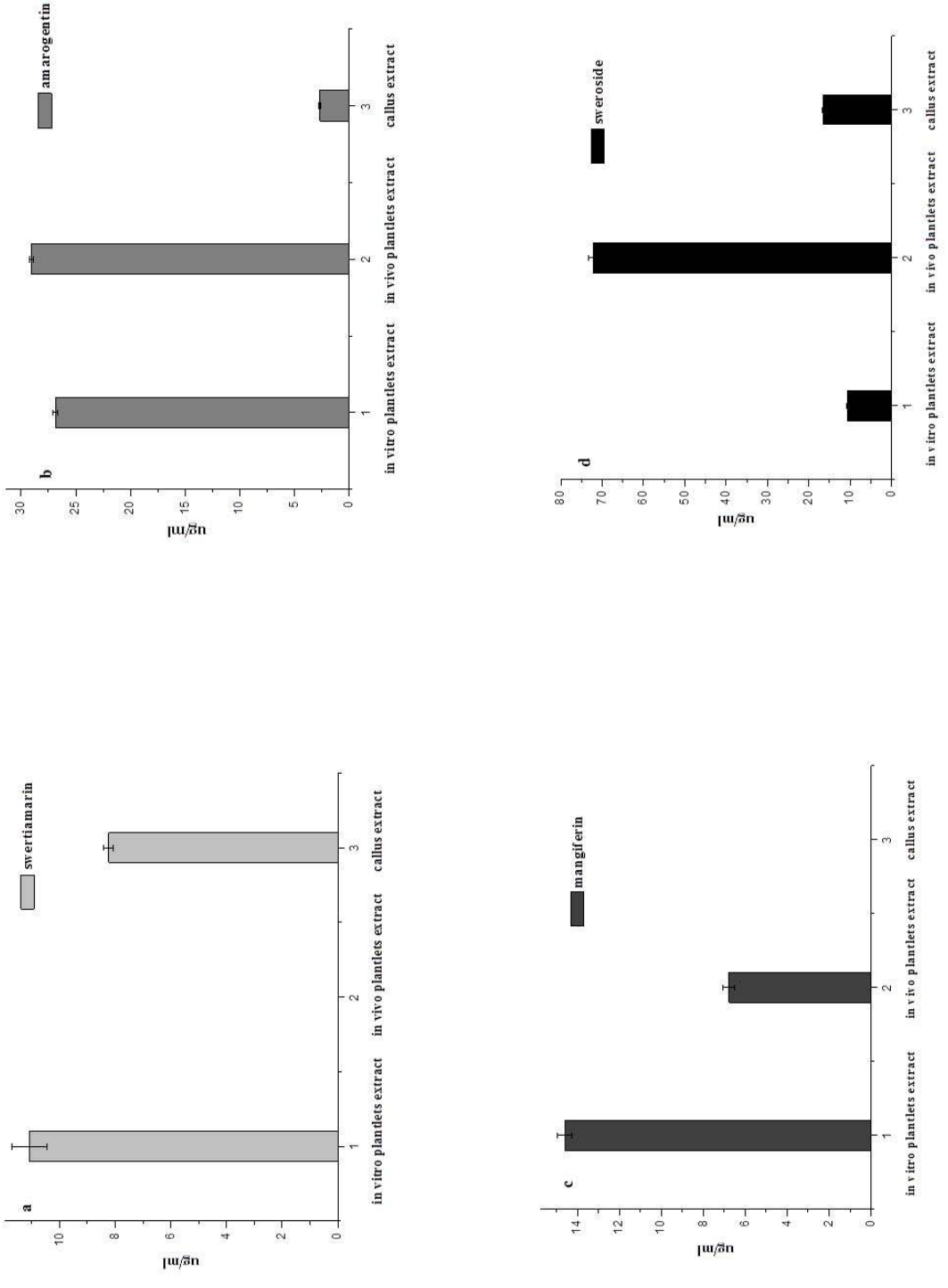


**Figure 8.** Amount of (a) swertiamarin; (b) amarogentin; (c) mangiferin; and (d) sweroside in *in vitro* plantlets, *in vivo* plantlets and callus extract of *S. chirayita*.

## 4. Conclusion

The current findings highlighted the organogenesis protocol *via* leaf explants of *S. chirayita* and the production of secondary metabolites. In addition, effects of chemical elements on the production of secondary metabolites. The protocol developed could be successfully employed for large-scale multiplication, conservation of germplasm and isolation of valuable secondary metabolites from *S. chirayita* an age old medicinal herb. The present study indicates the requirement to include an evaluation of bioactive secondary metabolite production when optimizing organogenesis protocol especially for highly valued medicinal plants.

| Chemical elements | In vitro plantlets | In vivo plantlets |
|---|---|---|
| As | ND | ND |
| Be | ND | ND |
| Ca | 5890 mg/kg | 3626 mg/kg |
| Cd | 3 mg/kg | ND |
| Co | 5 mg/kg | 4 mg/kg |
| Cr | 16.5 mg/kg | 14.5 mg/kg |
| Cu | 31 mg/kg | 21 mg/kg |
| Fe | 385 mg/kg | 870 mg/kg |
| Li | 8.5 mg/kg | 4 mg/kg |
| Mg | 1782 mg/kg | 1758 mg/kg |
| Mn | 396 mg/kg | 107.5 mg/kg |
| Mo | 22 mg/kg | 6 mg/kg |
| Ni | 2 mg/kg | 3.5 mg/kg |
| Pb | 47 mg/kg | 36 mg/kg |
| Sb | ND | ND |
| Se | 24 mg/kg | 26 mg/kg |
| Sr | 5 mg/kg | 22.5 mg/kg |
| Ti | 9.5 mg/kg | 66.5 mg/kg |
| Tl | ND | ND |
| V | 14 mg/kg | 63.5 mg/kg |
| ZN | ND | ND |

**Table 1.** Heavy metal contents in *in vitro* and *in vivo* plantlets of *S. chirayita* (mg/kg dry wt).

*ND- Not Detectable*

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