



# Optimizing nutrient media conditions for continuous production of shoot biomass enriched in major medicinal constituents, amarogentin and mangiferin of endangered medicinal herb, *Swertia chirayita*

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## Abstract

Biosynthesis and accumulation of Amarogentin and Mangiferin from shoot culture of endangered herb *Swertia chirayita* helped in rescuing its natural population along with continuous production of quality rich herbal material. Although, presence of Amarogentin and Mangiferin had already been reported, but such studies did not elaborate the significant developmental stages at two varying temperature ( $15 \pm 1$  °C and  $25 \pm 1$  °C) in shoot cultures of *S. chirayita*. Different developmental stages involved throughout from callus induction to complete regeneration of plant by using shoot cultures of *S. chirayita*, reveal different amounts of significant medicinal compounds having high pharmacological importance like bearing anti-diabetic and anti-cancerous properties. So in the present study, different developmental stages i.e. plant segment as leaf disc explants, initiation of callus formation, callus mass development, shoots primordial, manifold shoot formation and shoot elongation with complete growth were explored for accumulation of Amarogentin and Mangiferin. The Amarogentin content was  $4.72 \mu\text{g}/\text{mg}$  at  $15 \pm 1$  °C and  $4.41 \mu\text{g}/\text{mg}$  at  $25 \pm 1$  °C whereas Mangiferin content was  $15.54 \mu\text{g}/\text{mg}$  at  $15 \pm 1$  °C and  $9.70 \mu\text{g}/\text{mg}$  at  $25 \pm 1$  °C in leaf discs provided with the medium MS + 2,4D = 1 mg/L, 6BAP = 0.5 mg/L, TDZ = 0.5 mg/L, respectively. The accumulation of Amarogentin and Mangiferin started from callus cultures differentiating into shoots and reached to the detectable amount equivalent to actual leaf explants in fully grown shoots with content of  $5.79 \mu\text{g}/\text{mg}$  at  $15 \pm 1$  °C and  $5.35 \mu\text{g}/\text{mg}$  at  $25 \pm 1$  °C whereas  $15.56 \mu\text{g}/\text{mg}$  at  $15 \pm 1$  °C and  $13.15 \mu\text{g}/\text{mg}$  at  $25 \pm 1$  °C provided with the medium MS + IBA = 3 mg/L, KN = 1 mg/L, respectively. Maximum accumulation of bioactive compounds was observed in  $\approx 3$  months old in-vitro grown shoots at  $15 \pm 1$  °C wherein, the content of Amarogentin was  $\approx 8.51$  folds higher and Mangiferin was  $\approx 4.09$  folds higher than the  $\approx 3$  months old green house grown shoots. So, the in-vitro raised shoots of *S. chirayita* enriched with marker medicinal compounds would be utilized as ready to use raw material for pharmaceutical industries for herbal drug formulations and can be utilized to transfer under natural habitats for conserving its diminishing population.

**Keywords** Callus · Differentiation · Metabolites · Regeneration · Shoots

## Introduction

*Swertia chirayita* (Gentianaceae) is a medicinal herb, mainly present in the sub- temperate Himalayan region of India from Kashmir to Bhutan at an altitude range of 1200–3000 m. This herb is among the 32 most prioritized medicinal plants by (NMPB) National Medicinal Plant Board, Govt. of India (Kumar and Staden 2016). It mostly prefers moist shady areas and acidic soil for its growth (Sharma et al. 2011). *S. chirayita* is biennial plant reaching up to 1.5 m tall in height. Flowering generally occurs in the month of September with fruiting in the month of

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October. *S. chirayita*, also commonly called as chiretta, has been declared as an extremely endangered species due to over-exploitation of its herbal raw material as whole plants. The herb has been extensively used in various herbal drug formulations to relieve ailments like digestive diseases, liver disorder, inflammation, fever, malaria, asthma, ulcers, worms and sugar (Karan et al. 1999). *S. chirayita* is also known for its medicinal importance due to its pharmacological attributes like antidiabetic (Banerjee et al. 2000; Verma et al. 2008), antioxidant (Alam et al. 2009), antifungal (Laxmi et al. 2011), antibacterial, anti-inflammatory, anticancer and antiviral (Kar et al. 2003; Das et al. 2012; Verma et al. 2013) along with anti-hepatitis B virus activity as reported (Zhou et al. 2015). The medicinal importance of herb has been attributed to the presence of constituents such as Amarogentin and Mangiferin (Table 1) in addition to other metabolites like Gentiopicroin, Swertiamerin, Swerchirin, Sweroside, Amaroswerin etc. (Saha et al. 2006; Phoboo et al. 2013).

In addition to being almost extinct in its natural habitat in the Himalayan region with scarce availability in few pockets in Nepal region, the herbal drug industries or even the local communities trade adulterant in the market, which not only affect the quality and efficacy of herbal drugs but may also pose serious adverse health effects (Kumar and Staden 2016). Therefore, the only possibility to overcome limitations in ready availability of authentic herbal raw material from natural habitat is to develop shoot culture technologies so as to produce herbal raw material enriched for major chemical constituents. The only possibility to overcome this threat from natural surroundings of *S. chirayita* is to regulate the in-vitro tissue culture conditions for huge scope in generation of secondary metabolites and to save the genetic diversity of this species.

As per the development of its important secondary products through tissue cultures, need of complete knowledge

and understanding of production and its accumulation in varying developmental phases, is of prime requisite, so that particular phase can be identified that is uttermost worthy and reliable for in-vitro tissue culture along with the accumulation of Amarogentin and Mangiferin. Accumulation of Amarogentin and Mangiferin accounts to appear distinctively in shoots as well as in roots of *S. chirayita*. Whereas, Amarogentin mostly accumulates in the roots, shoots and Mangiferin accumulates in shoot cultures of flourished plant fields. The distinctive accumulation of Amarogentin and Mangiferin in shoots and roots of flourished plant fields stipulate biosynthesis of secondary metabolites which takes place in functional cell type. Though, factors responsible for biosynthesis of Amarogentin and Mangiferin in shoots and roots of flourished plant fields are unknown. In outer environment, herb is seasonal dependent and grows at very high altitude which leads to difficulty in understanding the biosynthesis and biology of metabolite production. However, in the cell culture condition, the controlled biological system wherein the regulation of different developmental stages can be controlled via changing the amount of growth regulators in the required media, leads to enormous production of pharmacologically important secondary metabolites (Ray and Jha 2001; Tanaka et al. 1995). Rapid multiplication of tissue culture of *S. chirayita* has been reported by Kumar et al. (2013), yet, there is no information regarding accumulation and biosynthesis of Amarogentin and Mangiferin in tissue culture *S. chirayita* at different developmental stages along with two different temperatures i.e.  $15 \pm 1$  °C and  $25 \pm 1$  °C.

Therefore, the spotting of different development phases in tissue culture of *S. chirayita* initiating from original explants as leaf discs, coming through different developmental stages as leaf discs de – differentiation into callus formation and further re- differentiation into shoot primordial formation and finally reaching to complete developed plant with elongated shoots, have been reported here. Dynamics

**Table 1** Pharmacological importance of Amarogentin and Mangiferin

| Sr. no. | Pharmacological importance (Amarogentin)     | References              | Pharmacological importance (Mangiferin) | References              |
|---------|--|-------------------------|---|-------------------------|
| 1       | Antioxidant                                  | Disasa et al. 2020      | Ovarian cancer                          | Zeng et al. 2020        |
| 2       | Inhibits liver cancer cell angiogenesis      | Zhang et al. 2020       | Used in osteoporosis                    | Sekiguchi et al. 2017   |
| 3       | Neurodegenerative disorder                   | Disasa et al. 2020      | Against gastric cancer                  | Du et al. 2017          |
| 4       | Inhibitory candidates against SARS-CoV-2     | Kar et al. 2020         | Reduces hyperglycaemia and obesity      | Imran et al. 2017       |
| 5       | Vascular metabolic effect                    | Potunuru et al. 2019    | Inhibits lung cancer                    | Rajendran et al. 2014   |
| 6       | Diabetic disorder                            | Niu et al. 2016         | Cure rheumatoid arthritis               | Luczkiewicz et al. 2014 |
| 7       | Anti-helminthic                              | Shubham and Mathur 2016 | Anti-inflammatory                       | Imran et al. 2017       |
| 8       | COX-2 inhibition used as (Anti-inflammatory) | Shukla et al. 2014      | Inhibits breast cancer                  | Imran et al. 2017       |
| 9       | Thromboembolic disorders                     | Yen et al. 2014         | Analgesic and antioxidant               | Dar et al. 2005         |
| 10      | Antileishmanial agent                        | Medda et al. 1999       | Anti-helminthic and Antiallergic        | García et al. 2003      |

of Amarogentin and Mangiferin accumulation in varying developmental tissue culture phases along with two different temperatures were reported. Moreover, a comparison has been made on the basis of secondary metabolite accumulation in the in-vitro and green house grown plant.

## Material and methods

### Plant material establishment

The cultures of *S. chirayita* were initiated by procuring field grown plants from Himalayan Forest Research Institute (HFRI), Shimla Himachal Pradesh, India (20°76'N, 67°12'E). Plants were authenticated by Dr. Y.S. Parmar University, India with UHF-Herbarium No. 13570 for carrying out further experimentation. Greenhouse grown plantlets of *S. chirayita* were maintained at JUIT, Waknaghat, Solan, India at (1400 m altitude) with controlled light conditions (1300–4700 W m<sup>-2</sup>) 25 ± 1 °C temperature, humidity (≈74%) and 14 h day and 10 h light photoperiod. For establishment of in-vitro grown plants of *S. chirayita*, plantlets were removed from the pots and shoot apices were surface sterilised using 0.3% Bavistin for 1–2 min along with 0.1% HgCl<sub>2</sub> for 30 s followed by 5–6 washing of autoclaved distilled water. Further, they were transferred to MS media supplemented with Benzylaminopurine (BAP) 2 mg/l, Kinetin (KN) 1 mg/l, Thidiazuron (TDZ) 0.5 mg/l and regularly sub cultured for its continuous maintenance (Murashige and Skoog 1962; Kumar et al. 2013).

### Initiation of callus induction

Leaf discs were excised from 4 weeks old in-vitro grown tissue cultured shoot of *S. chirayita* and further cultured in callus initiating medium for de-differentiating into callus (Fig. 1). Media which was used for de-differentiation, consisted of MS + 2,4 Dichlorophenoxyacetic acid (2,4-D) 1 mg/l, 6 Benzylaminopurine (BAP) 5 mg/l, Thidiazuron (TDZ) 0.5 mg/l, 0.8% agar–agar and 3% w/v sucrose and pH 5.7 (Fig. 1). MS medium was poured in 250 ml Erlenmeyer flask (Borosil) by dispensing 30 ml media in each culture bottle. The bottles were further incubated at plant tissue culture room at JUIT, India with controlled conditions (White fluorescent light (WFL) at 3000 lx intensity), 15 ± 1 °C and 25 ± 1 °C temperature, humidity (≈74%) with 16 h day and 8 h light photoperiod. A set of 6 explants per treatment was cultured on media bottles and every experiment was repeated for at least three times. After two weeks of explants inoculation, initiation of green colour callus was observed and after four weeks callus mass began to form.

### Plant complete regeneration and quantification of bioactive compounds through RP: HPLC

Further, callus mass was shifted to shoot generation media for re-differentiation. Basal composition of the nutrient medium was same, only changes were made in the growth hormone concentration. The medium used for re-differentiation consisted of MS + Indole-3 Butyric Acid (IBA) 3 mg/l and Kinetin (KN) 1 mg/l (Fig. 1). The estimation of Amarogentin and Mangiferin was analysed through RP-HPLC technique by using at least three replicates of each tissue culture samples that were taken from each developmental phase of tissue culture obtained from *S. chirayita* (Fig. 2, 3). For evaluation of Amarogentin and Mangiferin, RP-HPLC was carried out (Agilent 11,200 series) along with HPLC Pump C18 (5 µm) Waters column and Photodiode-Array detector (Waters 2996). Fresh sample was taken at each developmental stage further grounded by liquid nitrogen into fine powder and then suspended into 100 ml of 85% Methanol. Test sample was vortexed and left for the time being. Next day, the samples were sonicated for 2 s pulse at 30% amplitude for 10 min. After sonication, centrifugation was done at 10,000 rpm for 10 min. Supernatant was kept for further use and pellet was discarded. Same day, supernatant was filtered with 0.22 µm syringe filters. The filtrate was diluted to the level of 10× and inoculated in the column. The solvent system consist of Solvent A: (0.1% TFA trifluoro- acetic acid) and Solvent B: (70:30 Acetonitrile:Water mixture). The Amarogentin and Mangiferin were detected at 270 nm wavelength and the column was eluted in isocratic manner at 1.0 ml/min flow rate. The cycle time was at 25 °C for 30 min. The Amarogentin and Mangiferin were evaluated on the basis of their retention time with the standards obtained from Chromadex, Inc (Fig. 4).

### Statistical analysis













All experiments were repeated thrice and result obtained from all the experimentation was calculated by mean ± SD from the data.

## Results

### Quantification of bioactive compounds in different developmental phases

The accumulation of Amarogentin and Mangiferin in different development phases showed that leaf disc contains good content of Amarogentin which was observed to be 4.72 µg/mg at 15 ± 1 °C, 4.41 µg/mg at 25 ± 1 °C and Mangiferin content was 15.54 µg/mg at 15 ± 1 °C, 9.70 µg/mg at 25 ± 1 °C, respectively (Table 1). When the leaf disc

**Fig. 1** Different developmental phases in tissue culture shoots of *S. chirayita* from leaf disc used as explants. Scale bar = 1 cm

|    | Temperature   | 15±1°C   | 25±1°C  |
|----|---|--|---|
|    | Developmental Stage   | Leaf Segment   | Leaf Segment  |
| 1. | Plant Segment as leaf disc explants (0 Days)<br>(2,4D = 1mg/L, 6BAP = 0.5mg/L, TDZ = 0.5mg/L) |    |    |
| 2. | Callus initiation (1-15 Days)<br>(2,4D = 1mg/L, 6BAP = 0.5mg/L, TDZ = 0.5mg/L)                |    |    |
| 3. | Callus mass development (16-30 Days)<br>(2,4D = 1mg/L, 6BAP = 0.5mg/L, TDZ = 0.5mg/L)         |    |    |
| 4. | Shoots Primordia development (31-50 Days)<br>(BAP = 2 mg/L, KN = 1 mg/L)                      |    |    |
| 5. | Manifold shoot formation (51-65 Days)<br>(BAP = 2 mg/L, KN = 1 mg/L)                          |   |   |
| 6. | Shoot elongation with complete growth (66-80 Days)<br>(IBA = 3mg/L, KN = 1mg/L)               |  |  |

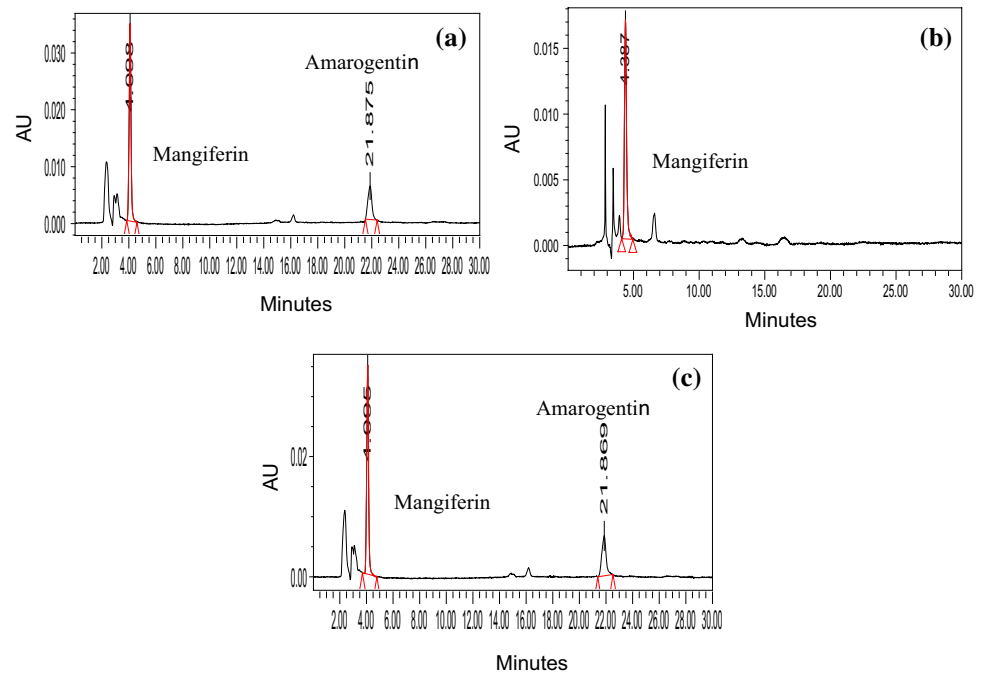
explants at two different temperatures initiated to de-differentiation and formed unorganized clump of cells i.e. callus after 10–15 days under optimized culture conditions, the content of Amarogentin and Mangiferin also started declining (Figs. 5, 6). After completion of four weeks when the explants were fully developed as callus mass under the incubation, the amount of Amarogentin was reduced to non detectable level to the 0.00 µg/mg at 15 ± 1 °C and 25 ± 1 °C. Mangiferin levels also showed reduction, but to a lesser extent as compared to Amarogentin and was detected within the range of 8.57 µg/mg at 15 ± 1 °C, 8.93 µg/mg at 25 ± 1 °C, respectively (Table 1).

As the callus mass formation occurred from the leaf explants, calli were further transferred for re-differentiation into shoots. After completion of five to six weeks under incubation, callus culture started differentiating into shoot primordial and after seven to eight weeks, they were fully developed into grown elongated shoots. The shoot

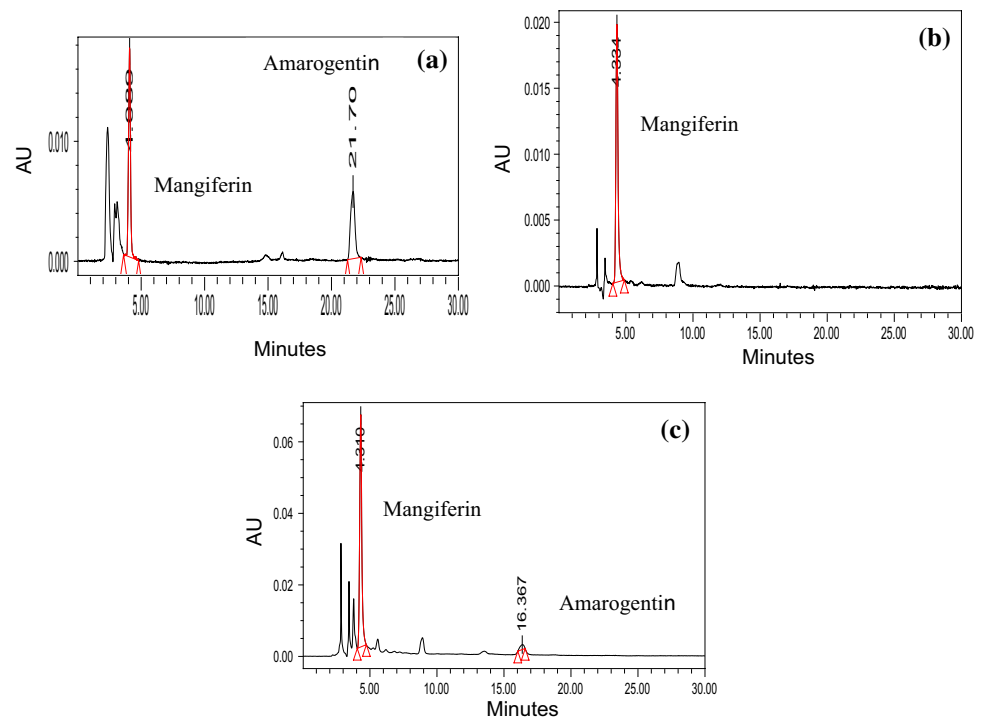
primordial showed accumulation of Amarogentin as well as Mangiferin. Surprisingly, amount of Amarogentin which was not detectable initially, also started to increase from callus mass to shoot primordia i.e. 0.89 µg/mg at 15 ± 1 °C and 0.96 µg/mg at 25 ± 1 °C, respectively. After completion of ten to twelve weeks, when the leaf disc explants converted to fully grown shoots, approximately equivalent amount of Amarogentin and Mangiferin were detected in both the temperatures i.e. 5.79 µg/mg at 15 ± 1 °C, 5.35 µg/mg at 25 ± 1 °C and 15.56 µg/mg at 15 ± 1 °C, 13.15 µg/mg at 25 ± 1 °C accordingly (Table 1). Difference observed in the accumulation of Amarogentin and Mangiferin content in shoots may be due to differences in their development and growth pattern Table 2.

As achieved from the above findings, maximum accumulation of Amarogentin (5.79 µg/mg) and Mangiferin (15.56 µg/mg) was observed in fully developed shoot cultures of *S. chirayita* (≈3 months old) at 15 ± 1 °C. So,

**Fig. 2** HPLC Chromatogram showing peaks of Amarogentin and Mangiferin at  $15 \pm 1$  °C. **a** Plant segment as leaf disc, **b** only Mangiferin is detected in callus mass cultures whereas Amarogentin is undetectable, **c** Complete grown shoots of *S. chirayita*

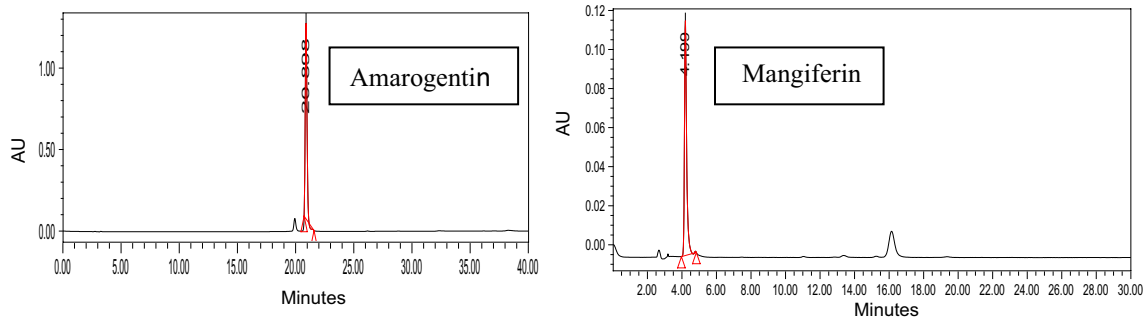


**Fig. 3** HPLC Chromatogram showing peaks of Amarogentin and Mangiferin at  $25 \pm 1$  °C. **a** Plant segment as leaf disc, **b** only Mangiferin is detected in callus mass cultures whereas Amarogentin is undetectable, **c** Complete grown shoots of *S. chirayita*

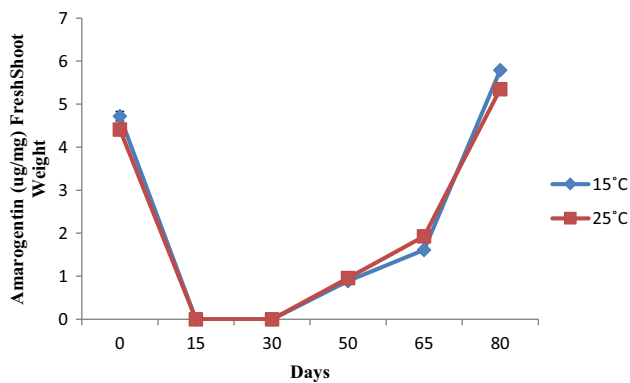


the comparison was carried out with the green house grown shoots ( $\approx 3$  months old) to quantify the content of Amarogentin and Mangiferin present in them (Fig. 7). So, through RP: HPLC quantification of biomarker compounds

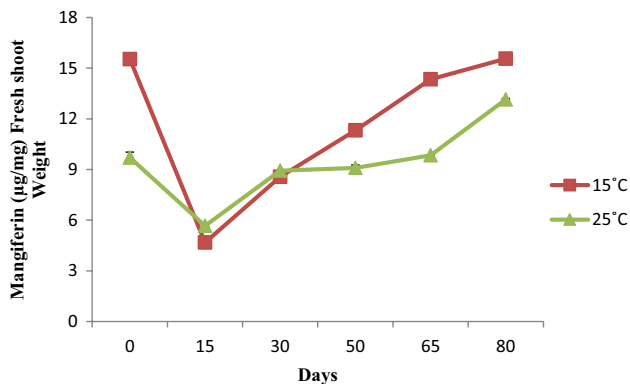
we concluded that after completion of  $\approx 3$  months, maximum accumulation of Amarogentin ( $0.68 \mu\text{g}/\text{mg}$ ) and Mangiferin ( $3.80 \mu\text{g}/\text{mg}$ ) was observed in the green house shoots of *S. chirayita*.



**Fig. 4** HPLC Chromatogram showing peaks of Standards Amarogentin and Mangiferin



**Fig. 5** Accumulation of Amarogentin in different developmental tissue culture phases of *S. chirayita* (1–6 as in Fig. 1). Error bar represents mean  $\pm$  SD as data recorded in triplicates



**Fig. 6** Accumulation of Mangiferin in different developmental tissue culture phases of *S. chirayita* (1–6 as in Fig. 1). Error bar represents mean  $\pm$  SD as data recorded in triplicates

## Discussion

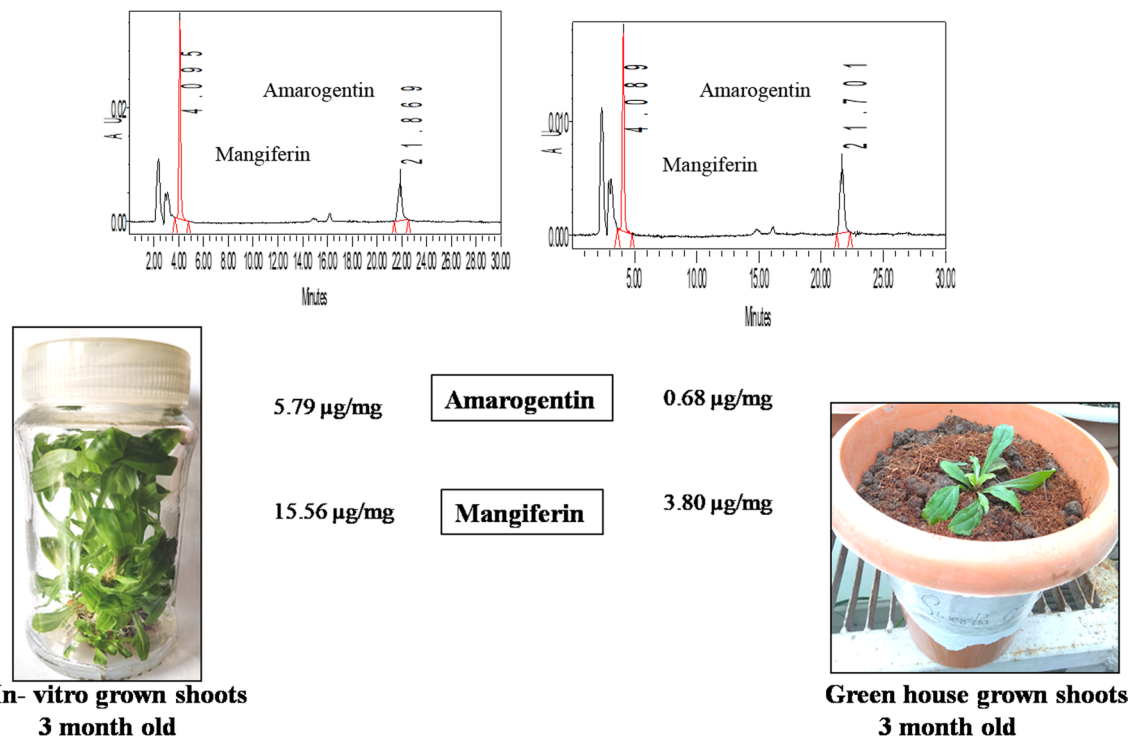
Above findings revealed that particular phase of development in tissue culture conditions is very crucial point for

the biosynthetic regulation of secondary metabolites. As during the process of development of plant, some stages are not very distinct, therefore we highlight here the different developmental stages starting from the explants up to the complete shoot development. The present work is in relevance with the study given by Sood and Chauhan 2009. Biosynthesis along with the accumulation of these important secondary metabolites differs from plant to plant and can be varied at different developmental stage as reported by various researchers (Sood and Chauhan 2009; Thiem and Krawczyk 2003; Grzegorzczuk et al. 2007; Aerts and De luca 1992). The Picroside -1 present in the leaf disc and shoot explants are used for fully grown plant growth whereas it is negligible in the root segment in *Picrorhiza kurroa* (Sood and Chauhan 2009). Similarly, in *Rubus chamaemorus* plants, ellagic acid is present as secondary metabolite which was found less in shoot and callus cultures (Thiem and Krawczyk 2003). The carnolic acid found in *Salvia officinalis* was only found in the shoot cultures and not in callus culture (Grzegorzczuk et al. 2007). In *Catheranthus roseus*, accumulation of vindoline is present in the shoot cultures but not in callus mass cultures (Aerts and De luca 1992). Similar results were observed in our case where most of the metabolites accumulate in shoot cultures and are in negligible amount in callus. The lack of Amarogentin in undifferentiated callus cultures and less amount of Mangiferin in callus culture of *S. chirayita*, might be due to absence of cell machinery programming along with the absence of balanced cell organization to occur in tissue culture condition for metabolite synthesis. As already reported by Kumar et al. 2015 wherein callus cultures of *S. chirayita* accumulates less amount of Amarogentin i.e. 0.05 and 0.09% of Mangiferin and further Kumar et al. 2014 reported negligible amount of Mangiferin and very less amount of Amarogentin, when they have already added Adenine sulphate for enhanced production of secondary metabolites, such findings were in relevance to our study, where we have reported 8.57  $\mu$ g/mg of Mangiferin and negligible amount of Amarogentin

**Table 2** Quantification of Amarogentin and Mangiferin in different development stages of in-vitro grown shoot cultures of *S. chirayita* at  $15 \pm 1$  °C and  $25 \pm 1$  °C

| Different developmental stages with time interval                                      | Amarogentin content (µg/mg)   |             | Mangiferin content (µg/mg) |              |
|--|---|-------------|----------------------------|--------------|
|  | 15 ± 1 °C   | 25 ± 1 °C   | 15 ± 1 °C                  | 25 ± 1 °C    |
|  | 0 Day<br>Plant segment (2,4D = 1 mg/L, 6BAP = 0.5 mg/L, TDZ = 0.5 mg/L) | 4.72 ± 0.11 | 4.41 ± 0.02                | 15.54 ± 0.05 |
| 0–15 Days<br>Callus initiation (2,4D = 1 mg/L, 6BAP = 0.5 mg/L, TDZ = 0.5 mg/L)        | 0.00 ± 0.00   | 0.00 ± 0.00 | 4.67 ± 0.03                | 5.67 ± 0.06  |
| 16–30 Days<br>Callus mass development (2,4D = 1 mg/L, 6BAP = 0.5 mg/L, TDZ = 0.5 mg/L) | 0.00 ± 0.00   | 0.00 ± 0.00 | 8.57 ± 0.03                | 8.93 ± 0.02  |
| 31–50 Days<br>Shoot primordiadevelopment (BAP = 2 mg/L, KN = 1 mg/L)                   | 0.89 ± 0.04   | 0.96 ± 0.02 | 11.32 ± 0.05               | 9.10 ± 0.17  |
| 51–65 Days<br>Manifold shoot formation (BAP = 2 mg/L, KN = 1 mg/L)                     | 1.61 ± 0.03   | 1.93 ± 0.03 | 14.34 ± 0.02               | 9.84 ± 0.01  |
| 66–80 Days<br>Shoot elongation with complete growth (IBA = 3 mg/L, KN = 1 mg/L)        | 5.79 ± 0.05   | 5.35 ± 0.05 | 15.56 ± 0.05               | 13.15 ± 0.04 |

\*Data has been recorded in triplicates (repeated three times) and presented as mean ± S D



**Fig. 7** Accumulation of Amarogentin and Mangiferin in *S. chirayita* plants grown in greenhouse and in-vitro conditions

at 15 °C in callus initiation stage. In the re-differentiation stage including shoot primordial along with fully grown shoots, the amount of Amarogentin and Mangiferin start increasing which can be attributed to the presence of enough amount of chloroplast which can be ruled out in case of callus mass as presence of less amount of chloroplast in this stage leads to reduction in the levels

of these secondary metabolites. However, the shut down or degraded biosynthesis pathway of Amarogentin in callus culture is yet to be understood completely. Accumulation of secondary metabolites reported by Kaur et al. 2019 in the leaf segment of *S.chirayita* quantified 0.68% of Amarogentin and 4.31% of Mangiferin however in our study, we have reported 4.72 µg/mg of Amarogentin and

15.54 µg/mg of Mangiferin at leaf segment stage. Shoot cultures of *S. chirayita* reported 1.03 µg/mg of Amarogentin and 2.99 µg/mg of Mangiferin using MS media supplemented with IBA 2 mg/l + KN 2 mg/l by Kumar et al. 2013, whereas in our case, we have reported  $\approx 5.62$  and  $\approx 5.20$  folds higher using MS media supplemented with IBA 3 mg/l + KN 1 mg/l in the fully grown and elongated shoots stages. These findings revealed that accumulation of Mangiferin occur favourably in both differentiated shoot cultures and in the de-differentiated callus cultures with lesser amount whereas, there is no biosynthesis of Amarogentin in de-differentiated callus mass. This opens up various traces to be explored in the biology behind Amarogentin synthesis in more significant details. The Amarogentin is secoiridoid glycoside which belongs to the monoterpene class of terpenoids and Mangiferin is xanthone c-glycoside (Pradhan et al. 2015) and organogenesis in cell culture conditions helps to induce monoterpene production as they lack to produce it in the un-differentiated callus formation (Shrivastava et al. 2006). At  $15 \pm 1$  °C, accumulation of Amarogentin and Mangiferin is found to be comparatively higher than at  $25 \pm 1$  °C which attributes that  $15 \pm 1$  °C is more suitable temperature for the tissue culture grown plants of *S. chirayita* (Fig. 4) as synthesis of biomarker compounds is influenced by many factors in which temperature is also one of the important factors (Kumar et al. 2015). Similarly, Picroside-1 content is found to be higher at  $15 \pm 1$  °C in comparison to  $25 \pm 1$  °C (Sharma et al. 2016). These results might reveal that low temperatures up regulated the gene expression for metabolite production which needs to be understood completely. It has been observed that when in-vitro developed shoots ( $\approx 3$  months old) were compared with the greenhouse shoots ( $\approx 3$  months old), the content of Amarogentin was  $\approx 8.51$  folds higher and Mangiferin was  $\approx 4.09$  folds higher than the greenhouse grown shoots (Fig. 7). So, the developed protocol in this study provides medicinal compounds rich shoots in short duration of time which could be used as an alternate to the field grown plants and contribute to abstain the reckless collection of this endangered medicinal herb from their natural habitat.

## Conclusion

This study covers the detailed exploration of different developmental stages in regard to Amarogentin and Mangiferin production which inferred accumulation of metabolites is tissue developmental stage-specific which was not reported so far in the shoot cultures of *S. chirayita*. The outcome of the present study revealed that shoot cultures of *S. chirayita* have the biosynthetic capability for the production of Amarogentin and Mangiferin in a shorter

duration of time under optimized culture conditions and help in the identification of optimum developmental stage like 3 months old shoots, which can be scaled up to a bioreactor level. Moreover, comparative analysis of in-vitro grown shoots with greenhouse shoots opened up the avenues for commercialization of this quality-rich planting material to pharmaceutical industries and for in-situ propagation by farmers.

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## Declarations

**Conflict of interest** Authors declare they have no conflict of interest.

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