

Micropropagation, antioxidant properties and phytochemical assessment of *Swertia corymbosa* (Griseb.) Wight ex C. B. Clarke: a medicinal plant

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Abstract *Swertia corymbosa* (Griseb.) Wight ex C. B. Clarke, a valuable medicinal plant, has been investigated for its regeneration potential using nodal explants. Out of a range of concentrations of cytokinins [6-benzyl adenine (BA), 6-furfurylaminopurine (Kn), 2-isopentenyl adenine (2iP), thidiazuron (TDZ), and zeatin (Z)] used as supplements with MS, BA at 4.40 μM concentration proved best for multiple shoot induction yielding 26.50 ± 0.26 shoots after 12 weeks of culture. Addition of low concentration of NAA (1.3 μM) in MS medium supplemented with the cytokinin BA (4.40 μM) favoured shoot multiplication. A mean number of 35.78 ± 0.81 shoots were produced per explant. Additive effect of BA (4.40 μM) in combination with Kn (4.64 μM) produced highest number of shoots (83.20 ± 4.29). Addition of GA_3 (1.4 μM) to the above medium not only favored shoot elongation but also enhanced the number of shoots (113.98 ± 3.80). The microshoots were rooted successfully on half-strength MS medium supplemented with 9.8 μM of IBA. The plantlets were successfully transferred to hardening medium containing vermiculite with 87 % survival rate. Screening of the antibacterial, antioxidant activity and estimation of total phenolic and flavonoid content of methanolic extracts of micropropagated plants were also carried out and compared with that of the wild-grown plants. In all the tests, methanolic extract from wild-grown plants showed higher antioxidant, antimicrobial activity, total phenolic and flavonoid content than in vitro propagated plants. The content of

secondary metabolites in wild-grown plants and in vitro propagated plants was determined by HPLC coupled with ESI-MS and the presence of loganic acid, swertiamarin, sweroside, gentiopicroside, isovitexin, amoroswertin, amarogentin, gentiacaulein, decussatin, and swertianin in the samples were confirmed. Gentiopicroside (40.726 mg/g) and swertianin (29.598 mg/g) were found to be the major compounds which may be responsible for the antimicrobial and antioxidant activities. The results of the present study confirmed the therapeutic potency of *S. corymbosa* used in the traditional medicine; in addition, the protocol for in vitro production developed in the present study could be applied for mass multiplication and for the conservation of germplasm.

Keywords Nodal explants · Plant growth regulators · In vitro propagation · Antioxidant · 2,2-Diphenyl-1-picrylhydrazyl · Antimicrobial activity · Xanthonoid · Gentiopicroside · Swertiamarin

Abbreviations

IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
NAA	α -Naphthaleneacetic acid
TDZ	Thidiazuron
BA	6-Benzyl adenine
Kn	6-Furfurylaminopurine
2-iP	2-Isopentenyladenine
GA_3	Gibberellic acid
Z	Zeatin
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ABTS	2,2'-Azinobis (3-ethylbenzothiozoline-6-sulfonic acid) diammonium salt
TPTZ	2,4,6-Tripyridyl-S-triazine
EDTA	Ethylenediamine tetraacetic acid
HPLC	High performance liquid chromatography

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HPLC–ESI-MS High performance liquid chromatography-electrospray ionization-mass spectrometry

Introduction

The genus *Swertia* (*Gentianaceae*), commonly known as ‘chirayata’, comprises about 170 species and is widely used as herbal medicine in the treatment of diabetes (Chhetri et al. 2005; Li et al. 2004; Tian et al. 2010). It is also used as blood purifier in liver diseases. The anti-inflammatory, anti-cancerous, anti-pyretic, hypoglycemic, anti-fungal and anti-bacterial activities were demonstrated by Brahmachari et al. (2004). Xanthonoids are the most abundant class of secondary metabolites in this genus; particularly the oxygenated xanthenes have been reported to exhibit numerous pharmacological activities (Brahmachari et al. 2004; Liu et al. 2006).

Swertia corymbosa (Griseb.) Wight ex C.B. Clarke is commonly known as Shirattakuchi among the Irula tribe. Medicinal properties of *S. corymbosa* are not well clarified. Like in some other species of the genus *Swertia*, its Xanthonoids, Decussatin (1), Gentiacaulein (2) and Swertianin (3) 1, 8-dihydroxy-2, 6-dimethoxyxanthone (4) and two new xanthenes 8-hydroxy-1, 2, 4, 6-tetramethoxyxanthone (5) 1, 2, dihydroxy-6-methoxyxanthone-8-*O*- β -D-xylopyranosyl (6) were isolated and its anti-inflammatory and anti-nociceptive activities were evaluated by Mahendran et al. (2013). *Swertia corymbosa* is propagated conventionally by seeds. However, poor germination potential restricts its multiplication. Propagation through tissue culture offers a viable alternative for multiplication of this species and for ex situ conservation. The objective of this study is to develop an effective in vitro regeneration protocol, to analyze the secondary metabolites and to evaluate the antioxidant, antimicrobial activity of both wild-grown and in vitro regenerated plants.

Materials and methods

Establishment of aseptic culture

The plant material was collected from the natural habitat of Vellingiri hills, Coimbatore (Tamilnadu), at an altitude of 1,850 msl (mean sea level). The plant was authenticated by Botanical Survey of India, Coimbatore. The herbarium is deposited in the Department Botany, Bharathiar University, Coimbatore (Accession Number: 006144). After removing the leaves, the nodal segment (1 cm) was excised and washed thoroughly under running tap water followed by 1 % (v/v) solution of teepol treatment (Reckitt

Benckiser—India Ltd., India) for 5 min. The explants were rinsed repeatedly with sterile distilled water and then treated with a fungicide Bavistin (1 %) for 10 min and washed thoroughly in sterile distilled water. All subsequent operations were carried out inside a laminar air-flow cabinet (Clean Air System- India). The nodal cuttings were given a quick rinse (30 s) in 70 % ethanol before surface sterilizing with 0.01 % (w/v) mercuric chloride solution for 5 min. The nodal explants was washed thoroughly with sterile water and cultured upright with the basal end of the node inserted a few millimeters into the culture medium.

Media and culture conditions

MS (Murashige and Skoog 1962) medium with 3 % (w/v) sucrose (Hi Media Laboratories, India) supplemented with different plant growth regulators in various concentrations was used in the experiment. The pH of the medium was adjusted to 5.8 using 1 N NaOH or 0.1 N HCL and the medium was solidified with 0.8 % (w/v) agar (Hi Media Laboratories, India) before autoclaving at 121 °C for 15 min. The shoot cultures were incubated in culture room at 25 \pm 2 °C under 16/8 h (light/dark) cycle with a light intensity of 50 $\mu\text{mol}^{-2} \text{s}^{-1}$ supplied by cool white fluorescent lamp (2 tubes, 40 Watt, Philips, India) and with 75–80 % relative humidity.

Multiple shoot induction

The nodal explant with axillary bud (single node with two axillary buds; 2-month-old explant was collected from natural habit) was cultured on MS medium supplemented with different plant growth regulators in various concentrations such as BA (1.10–8.80 μM), Kn (1.15–9.20 μM), 2-iP (1.01–8.12 μM), Z (1.10–9.10 μM), and TDZ (1.10–9.00 μM) either individually or in combination with NAA (1.30 μM) for multiple shoot induction. The additive effects of BA (1.10–8.80 μM) in combination with Kn (2.32 or 4.64 μM) were also tested. GA₃ at concentration of 1.4 μM was added to the above media to test its effect on shoot production and shoot elongation. Cultures were subcultured onto fresh media every 4 weeks, maximum three times. The number of shoots per explant and shoot length, the number of roots and root length were recorded after 12 weeks of culture.

The shoot multiplication

The shoots were subcultured regularly at 90-days interval. The obtained micro-shoots were cut into segments (0.5 cm) each with single node and subcultured on MS medium containing BA (4.40 μM) alone or in combination with

NAA (1.3 μM) Kn (4.64 μM) or Kn and GA_3 (1.4 μM). Totally five subcultures were made. The number of shoots and shoot length per explant were recorded at every subculture.

Rooting and transplantation

For in vitro root induction, the microshoots (4–5 cm long) were transferred to half-strength MS medium supplemented with IAA (2.8, 5.6 or 11.2 μM), IBA (2.4, 4.9 or 9.8 μM) or NAA (2.6, 5.3 or 10.7 μM). After 12 weeks, the rooted microshoots were washed thoroughly and transferred to the plastic pots (5 cm) containing autoclaved vermiculite or vermiculite, soil, and perlite in the ratio of 1:1:1 and maintained in culture room. The plantlets were covered with polyethylene bags to maintain high humidity and irrigated with sterile distilled water. After 2 months, 3–4 small holes were made in the bag and maintained for two more months, and the plantlets were hardened.

Preparation of methanol extracts

The biomass from in vitro propagated plants (4-week-old culture, MS medium supplemented with BA (4.40 μM) shoots were collected) and wild-grown plants (aerial parts) were washed under tap water and dried in oven at 60 °C for 2 days. The material was powdered using electric blender and stored in clean labeled airtight bottles. The powder (100 g) was extracted by maceration in 300 ml of methanol (100 %) for 3 days with frequent agitation. The mixture was filtered through Whatman No. 1 filter paper and the filtrate was concentrated and dried in Petridishes at 60 °C in the oven.

Estimation of total phenolics (TPC) and total flavonoid content (TFC)

The total phenolic content of the extracts was determined and calculated as gallic acid equivalent (GAE) in mg/g DW from the calibration curve according to method described by Siddhuraju and Becker (2003). The total flavonoid content of sample extract was determined following a colorimetric method and values were expressed as mg/g rutin equivalent (RE) of extract according to the method described by Zhishen et al. (1999).

In vitro antioxidant activity

The free radical scavenging activity of the *S. corymbosa* methanol extract of wild-grown plants and in-vitro propagated plants was evaluated using DPPH (Blois 1958),

ABTS⁺ cation radical (Re et al. 1999), ferric reducing antioxidant power (FRAP) activity (Pulido et al. 2000) and metal chelating activity (Dinis et al. 1994).

Antimicrobial activity

Test bacteria

The antibacterial activity of the plants collected from natural habitat and in vitro regenerated plants were evaluated against six pathogenic bacteria such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Streptococcus pneumoniae* (ATCC 33400), *Klebsiella pneumoniae* (ATCC 10031), and *Bacillus subtilis* (ATCC 6633) procured from the Institute of Microbial Technology (IMTECH) Chandigarh, India. All the strains were stored in the appropriate medium before use.

Disc diffusion method

Disc diffusion method (Joshi et al. 2010) was used for the evaluation of antibacterial activity of extracts using 100 μl of suspension containing 10^8 CFU/ml of bacteria spread on the inoculated agar. Empty sterilized discs were impregnated with 20 μl of diluted extracts with test concentrations (25, 50, 75 and 100 $\mu\text{g/ml}$) and the same volume (20 μl) of methanol was used as control. Gentamicin (10 $\mu\text{g/disc}$) was used as a positive reference standard to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition (mm) against the test organisms. The experiments were repeated in triplicate and the results were expressed as average values.

HPLC–ESI-MS determination of bioactive compounds

A Beckman 125 HPLC instrument (Beckman, USA) with an ultraviolet/visible detector (UV/Vis) was coupled to an ion trap mass spectrometer with an ESI interface. The chromatogram was recorded at 254 nm. A 150 \times 4.6 mm, i.d., 5 μm particle size, ODS-C18 column (Diamonsil, Dikma, China) with the column temperature set at 35 °C was used for separation. The injection volume was 10 μl , and the elution was performed at a flow rate of 0.8 ml/min using a mixture of 1 % acetic acid (solvent A) and acetonitrile (solvent B) as mobile phases. (B). The gradient elution was as follows: 0–8 min, 2–15 % (B), 8–20 min, 15 % (B), 20–50 min, and 15–100 % (B).

Mass analyses were performed using an ESI interface in the positive ion mode. The data were acquired in the full-scan and MS/MS² scanning modes. The optimized

instrumental parameters were set as follows. Positive mode: desolvation temperature 250 °C; source temperature, 120 °C; capillary voltage, 1.2 kV; cone voltage, 30 V; desolvation gas (N₂) flow rate, 900 L/h; cone gas (He) flow rate, 40 L/h; and scan range, m/z 100 amu to 1000 amu. The MS/MS2 spectra were acquired using collision energy of 40 V. The components of secondary metabolites in the samples were identified on the basis of characteristic on the retention time, EI-MS spectra, and spiking the sample with the standard and were quantified by using an external standard.

Statistical analysis

The experiments were repeated thrice and each set had ten replicates. The significance of differences among means was assessed using Duncan's multiple range test at $P < 0.05$ (ANOVA). The results are expressed as a mean \pm SE of three experiments. Data of the antioxidant, total phenolic and flavonoid assays were expressed as the mean \pm standard deviation (SD) of three independent measurements. Correlation analysis was performed between phenolics and flavonoids with antioxidant activity using Pearson correlation two-tailed. The results were analyzed statistically using SPSS Version 17 (SPSS Inc., Chicago, USA).

Results and discussion

Effect of cytokinins

The nodal explants cultured on MS medium without any growth regulators failed to produce shoot and the explants eventually died. In the presence of cytokinin, the explant responded positively in producing multiple shoots (Fig. 1a). It is known that cytokinins regulate many cellular processes, but the control of cell division is central in growth and development of varieties of medicinal plants (Faisal et al. 2006; Anis et al. 2009). Among the cytokinins tested, BA at concentration of 4.40 μ M proved to be the most effective for multiple shoot induction (Table 1) (Fig. 1b). BA was found to have a greater influence than either Kn or 2iP or Z or TDZ for axillary bud initiation and proliferation, which is well supported by earlier findings in many medicinal plants including *Ocimum basilicum* (Siddique and Anis 2008), *Lawsonia inermis* (Ram and Shekhawat 2011), and *Vitex negundo* (Ahmad and Anis 2011; Ahmad et al. 2013). NAA (1.3 μ M) in combination with cytokinins also favored shoot formation. However, the response for multiple shoot induction varied (Table 2). Among the different combinations, maximum number of shoots were produced in MS supplemented with BA

Fig. 1 The initiation, multiplication, elongation and rooting of *S. corymbosa* shoots in vitro. **a** Initiation of multiple shoots from nodal explant. (Bar 1.0 cm). **b** Multiple shoot induction in MS medium with 4.40 μ M of BA (Bar 1.0 cm). **c** Multiple shoot induction in MS medium with 4.40 μ M of BA in combination with 4.64 μ M of Kn (Bar 1.5 cm). **d** Elongated shoot on MS medium supplemented with BA at 4.40 μ M, Kn at 4.64 μ M and GA₃ at 1.4 μ Me. **e** Multiple shoot induction on MS medium supplemented with BA at 4.40 μ M, Kn at 4.64 μ M and GA₃ at 1.4 μ M. **f** Development of both root and shoot on MS + BA (4.40 μ M) + Kn (4.64 μ M) + GA₃ (1.4 μ M) after 90 days. **g** Micro shoot rooted in half-strength MS medium with 9.8 μ M IBA. **h** Base line callus on rooting medium containing 2.6 μ M of NAA. **i** Plantlets were covered by polyethylene bags and maintained inside the culture room for acclimatization. **j** Hardening

(4.40 μ M) and NAA (1.3 μ M). A stimulatory effect of BA in combination with NAA has been reported in various medicinal plant species such *Nyctanthes arbor-tristis* (Siddique et al. 2006), *Mucuna pruriens* (Faisal et al. 2006), *Gentianella austriaca* (Vinterhalter et al. 2008), *Swertia chirata* (Wang et al. 2009; Balaraju et al. 2009), and *V. negundo* (Ahmad et al. 2013).

Additive effect of different cytokinins on multiple shoot induction

A twofold increase in the number of the shoots (83.20 ± 4.29) was observed when the nodal explants were cultured on MS medium supplemented with BA (4.40 μ M) and Kn (4.64 μ M) compared with (BA alone or BA + NAA) (Table 3; Fig. 1c). However, the elongation of these shoots was not satisfactory. Addition of GA₃ (1.44 μ M) to BA +Kn medium resulted in significant growth of shoot (10.21 ± 0.57 cm) (Fig. 1d) and further increase in the number of microshoots (113.98 ± 3.80) (Table 3; Fig. 1e). Additive effect of BA and Kn in promoting shoot initiation has been reported early in *Acacia catechu* (Indra et al. 2000), *Spilanthes paniculata* (Mahendran et al. 2006), *S. chirata* (Chaudhuri et al. 2007; Balaraju et al. 2009), *Bauhinia racemosa* (Rajanna et al. 2011), and *Streblus asper* (Gadidasu et al. 2011). The positive role of GA₃ in promoting in vitro shoot development has also been reported in *Gentiana corymbifera* (Morgan et al. 1997) and *G. triflora* (Zhang and Leung, 2002).

The crucial steps of micropropagation are the rooting and acclimatization of in vitro plantlets. Following 90 days of culture on proliferation medium (BA + Kn or BA + Kn + GA₃), some of the *S. corymbosa* shoots spontaneously formed roots. Among the different combinations, 4.64 μ M Kn + 4.40 μ M BA +1.4 μ M GA₃ proved to be the most effective for root induction (Table 3) (Fig. 1f). There are also technical advantages of this rooting as it eliminates the additional in vitro rooting step and combines the steps of rooting of regenerates. This means

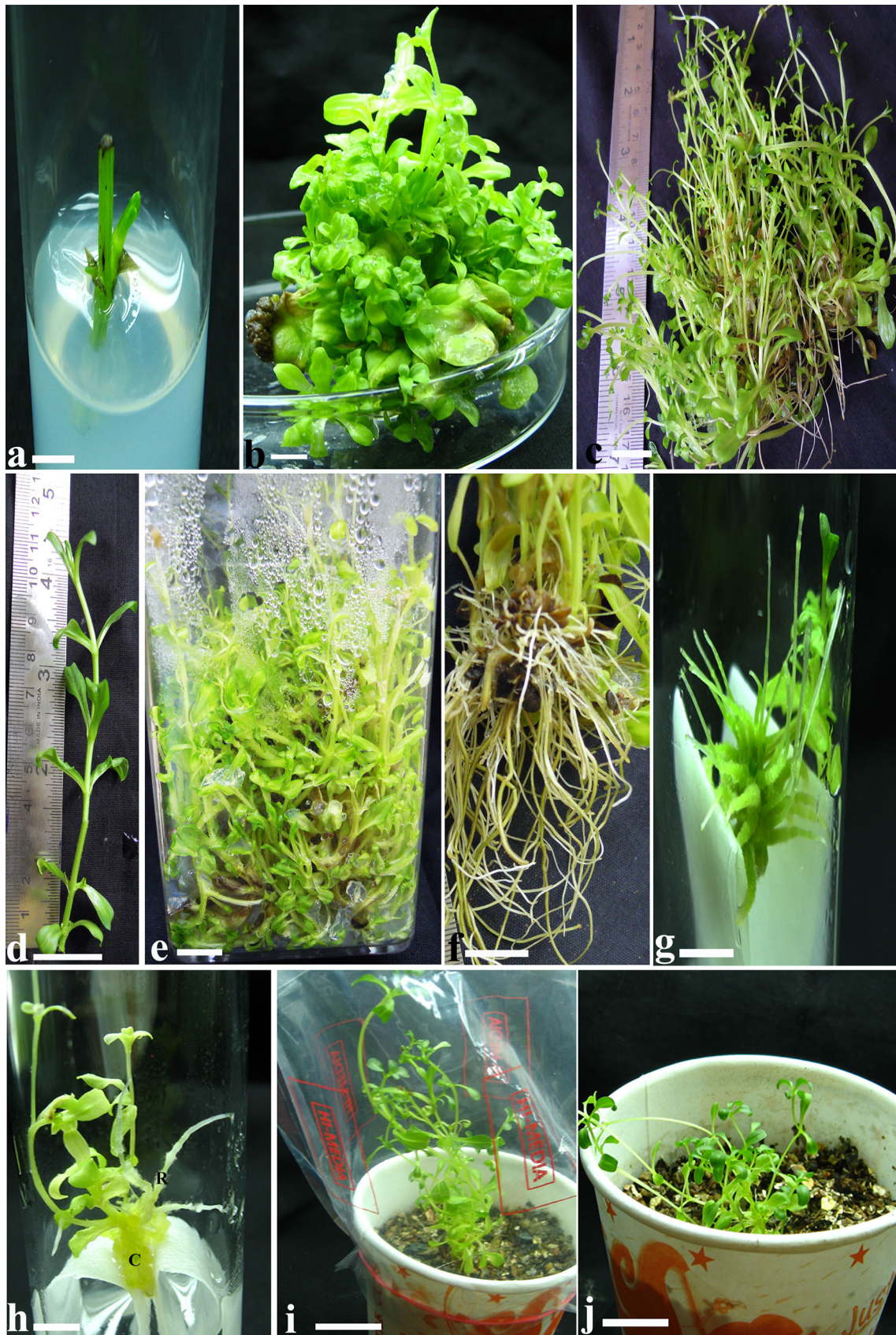


Table 1 Effect of different cytokinins on multiple shoot induction from nodal explant of *Swertia corymbosa* in vitro

BA ($\mu\text{M/l}$)	Kn ($\mu\text{M/l}$)	Z ($\mu\text{M/l}$)	TDZ ($\mu\text{M/l}$)	2ip ($\mu\text{M/l}$)	No. of shoots/explant	Shoot length (cm)
	–	–	–	–	–	–
1.10	–	–	–	–	4.20 \pm 0.13 ^{i,j}	3.66 \pm 0.66 ^{a,b}
2.20	–	–	–	–	9.30 \pm 0.15 ^g	3.00 \pm 0.79 ^{b,c}
3.30	–	–	–	–	16.30 \pm 0.30 ^d	3.41 \pm 0.54 ^{a,b}
4.40	–	–	–	–	26.50 \pm 0.26 ^a	3.01 \pm 0.02 ^{b,c}
6.60	–	–	–	–	18.50 \pm 0.26 ^c	3.06 \pm 0.02 ^{b,c}
8.80	–	–	–	–	15.30 \pm 0.26 ^e	3.00 \pm 0.40 ^{b,c}
–	1.15	–	–	–	3.20 \pm 0.13 ^{k,l}	3.00 \pm 0.00 ^{b,c}
–	2.32	–	–	–	5.30 \pm 0.15 ^h	3.11 \pm 0.77 ^{b,c}
–	3.45	–	–	–	10.10 \pm 0.31 ^f	2.15 \pm 0.24 ^{c,d}
–	4.64	–	–	–	15.80 \pm 0.32 ^{d,e}	2.52 \pm 0.30 ^c
–	6.90	–	–	–	20.10 \pm 0.27 ^b	3.20 \pm 0.49 ^b
–	9.20	–	–	–	15.40 \pm 0.30 ^e	3.42 \pm 0.23 ^{a,b}
–	–	1.10	–	–	4.60 \pm 0.16 ⁱ	3.63 \pm 0.20 ^{a,b}
–	–	2.20	–	–	3.00 \pm 0.00 ^l	3.61 \pm 0.31 ^{ab}
–	–	3.30	–	–	3.70 \pm 0.15 ^{j,k}	3.55 \pm 0.30 ^{a,b}
–	–	4.50	–	–	4.30 \pm 0.15 ^{i,j}	2.81 \pm 0.28 ^{b,c}
–	–	6.80	–	–	3.50 \pm 0.16 ^{k,l}	3.52 \pm 0.79 ^{a,b}
–	–	9.10	–	–	2.50 \pm 0.16 ^m	2.84 \pm 0.84 ^{b,c}
–	–	–	1.10	–	2.20 \pm 0.13 ^m	3.66 \pm 0.66 ^{a,b}
–	–	–	2.20	–	3.20 \pm 0.13 ^{k,l}	3.00 \pm 0.79 ^{b,c}
–	–	–	3.30	–	3.60 \pm 0.16 ^{k,l}	3.41 \pm 0.54 ^{a,b}
–	–	–	4.50	–	4.30 \pm 0.15 ^{i,j}	3.01 \pm 0.02 ^{b,c}
–	–	–	6.80	–	5.30 \pm 0.15 ^h	3.06 \pm 0.02 ^{b,c}
–	–	–	9.00	–	4.35 \pm 0.15 ^{i,j}	3.00 \pm 0.40 ^{b,c}
–	–	–	–	1.01	2.45 \pm 0.16 ^m	3.11 \pm 0.57 ^{b,c}
–	–	–	–	2.03	3.10 \pm 0.13 ^{k,l}	3.41 \pm 0.54 ^{a,b}
–	–	–	–	3.04	4.25 \pm 0.45 ^{i,j}	3.00 \pm 0.69 ^{b,c}
–	–	–	–	4.06	5.30 \pm 0.15 ^h	3.00 \pm 0.10 ^{b,c}
–	–	–	–	6.09	9.70 \pm 0.65 ^g	2.42 \pm 0.40 ^c
–	–	–	–	8.12	12.65 \pm 0.45 ^f	2.84 \pm 0.92 ^{b,c}

Values represent mean \pm SE of three repeated experiments each with ten replications. Means in a column with the different letter (superscript) are significantly different according to DMRT ($P < 0.05$)

that the cost and time required for clonal multiplication are greatly reduced. The remaining shoots were transferred to different rooting media.

The effect of subcultures was also evaluated on shoot multiplication rate by transferring the regenerated nodal explants to MS medium supplemented with different cytokinins. The highest number of shoots and shoot length was achieved in the first four subcultures beyond which a decline in multiplication rate was observed (Table 4). The rate of shoot multiplication is known to differ from species to species during the subculture. This enhanced shoot multiplication by subsequent cultures substantiates the earlier reports on *Capsicum annuum* (Siddique and Anis 2006), *Cassia angustifolia* (Siddique and Anis 2007), and *O. basilicum* (Siddique and Anis 2008).

Rooting and acclimatization

The success of micropropagation relies on efficient rooting of regenerated shoots and survival of plantlets under field conditions. IBA is a common auxin used for inducing rooting in several *Gentianaceae* plant species in *S. chirata* (Chaudhuri et al. 2007), *G. austriaca* (Vinterhalter et al. 2008). Among the tested auxins, IBA proved to be the most effective for root induction (Table 5). Callus intervening root formation was observed in shoots cultured on medium containing NAA (Fig. 1h). This differential requirement for auxin type may be influenced by the level of endogenous auxins in cultured shoots. Similar results have been observed for root initiation in the in vitro shoots of *Boswellia serrata* (Nikam et al. 2013) and *B. ovalifoliolata*

Table 2 Effect of different cytokinins in combination with NAA (1.3 μM) on multiple shoot induction from nodal explant of *Swertia corymbosa* in vitro

BA (μM)	Kn (μM)	Z (μM)	TDZ (μM)	2ip (μM)	No. of shoots/explant	Shoot length (cm)
1.10	–	–	–	–	5.76 \pm 0.34 ^e	3.53 \pm 0.12 ^{a,b}
2.20	–	–	–	–	10.25 \pm 0.12 ^{d,e}	3.09 \pm 0.46 ^{b,c}
3.30	–	–	–	–	21.30 \pm 0.53 ^{b,c}	3.32 \pm 0.74 ^{b,c}
4.40	–	–	–	–	35.78 \pm 0.81 ^a	3.29 \pm 0.62 ^{a,b}
6.60	–	–	–	–	23.43 \pm 0.36 ^b	3.12 \pm .62 ^{bc}
8.80	–	–	–	–	18.34 \pm 0.66 ^c	3.10 \pm 0.40 ^{b,c}
–	1.15	–	–	–	4.90 \pm 0.63 ^{e,f}	3.09 \pm 0.00 ^{b,c}
–	2.32	–	–	–	8.98 \pm 0.11 ^e	3.12 \pm 0.17 ^{b,c}
–	3.45	–	–	–	12.64 \pm 0.21 ^d	2.16 \pm 0.24 ^{b,c}
–	4.64	–	–	–	19.89 \pm 0.32 ^c	2.46 \pm 0.20 ^{b,c}
–	6.90	–	–	–	23.65 \pm 0.32 ^b	3.22 \pm 0.45 ^b
–	9.20	–	–	–	18.12 \pm 0.42 ^c	3.48 \pm 0.17 ^{a,b}
–	–	1.1	–	–	3.34 \pm 0.22 ^g	3.71 \pm 0.41 ^{a,b}
–	–	2.2	–	–	2.00 \pm 0.09 ^{g,h}	3.54 \pm 0.26 ^{a,b}
–	–	3.3	–	–	3.12 \pm 0.86 ^g	3.73 \pm 0.11 ^{a,b}
–	–	4.5	–	–	3.79 \pm 0.98 ^g	2.39 \pm 0.32 ^{b,c}
–	–	6.8	–	–	3.50 \pm 0.21 ^g	3.66 \pm 0.42 ^{a,b}
–	–	9.1	–	–	2.24 \pm 0.56 ^{g,h}	2.83 \pm 0.84 ^{bc}
–	–	–	1.1	–	2.29 \pm 0.12 ^{g,h}	3.75 \pm 0.34 ^{a,b}
–	–	–	2.2	–	3.09 \pm 0.34 ^g	3.00 \pm 0.00 ^{b,c}
–	–	–	3.3	–	3.97 \pm 0.14 ^g	3.59 \pm 0.11 ^{a,b}
–	–	–	4.5	–	4.39 \pm 0.91 ^{e,f}	3.00 \pm 0.13 ^{b,c}
–	–	–	6.8	–	4.92 \pm 0.11 ^{e,f}	3.10 \pm 0.79 ^{b,c}
–	–	–	9.0	–	4.94 \pm 0.22 ^{e,f}	3.00 \pm 0.92 ^{bc}
–	–	–	–	1.01	2.12 \pm 0.34 ^{g,h}	3.09 \pm 0.92 ^{b,c}
–	–	–	–	2.03	3.11 \pm 0.70 ^g	3.48 \pm 0.44 ^{a,b}
–	–	–	–	3.04	4.61 \pm 0.07 ^{e,f}	3.00 \pm 0.23 ^{b,c}
–	–	–	–	4.06	5.53 \pm 0.22 ^e	3.00 \pm 0.45 ^{bc}
–	–	–	–	6.09	7.50 \pm 0.65 ^e	2.43 \pm 0.27 ^{b,c}
–	–	–	–	8.12	9.12 \pm 0.29 ^{d,e}	2.87 \pm 0.21 ^{b,c}

Values represent mean \pm SE of three repeated experiments each with 10 replications. Means in a column with the different letter (superscript) are significantly different according to DMRT ($P < 0.05$)

(Chandrasekhar et al. 2005) cultured on the medium containing IBA and NAA, alone or in combination. The plantlets were transferred to the potting medium containing vermiculite or vermiculite, soil and perlite (1:1:1). A good survival rate of 87 % was achieved in pots containing vermiculite alone (Fig. 1i, j).

Estimation of total phenolics (TPC) and total flavonoid content (TFC)

Our results showed the twofold higher total phenolic and flavonoid contents in wild-grown plants compared to in vitro propagated plants (Table 6). Wild-grown plants gave higher

phenolic compounds and total flavonoid content probably due to a higher stress associated with their growth conditions. Otherwise, in vitro cultured seem to be under less stress producing lower amounts of phenolic and flavonoid compounds (secondary metabolites). In fact, in vitro cultured plants were grown under controlled light, temperature and nutrients. Similarly, early researchers found that lower amounts of phenolic compounds (secondary metabolites) were observed in in vitro cultured samples of *Poliomintha glabrescens* (Garcia-Perez et al. 2012), *Hypericum* spp. (Danova et al. 2012), *Cichorium pumilum* (Khateeb et al. 2012) *Hypericum undulatum* (Rainha et al. 2013), and *Melissa officinalis* (Barros et al. 2013).

Table 3 Effect of cytokinins with GA₃ on multiple shoot induction and elongation of *S. corymbosa* in vitro

BA (μM)	Kn (μM)	GA ₃ (μM)	No. of shoots	Shoot length (cm)	Total number of root number	Root length (cm)
1.10	2.32	–	17.20 ± 0.20 ⁱ	3.59 ± 0.25 ^h	4.88 ± 0.57 ^o	5.34 ± 1.17 ^{d,e}
2.20	2.32	–	20.10 ± 0.23 ^h	4.40 ± 0.51 ^{f,g}	9.34 ± 0.33 ⁿ	5.00 ± 0.87 ^f
3.30	2.32	–	33.30 ± 1.30 ^f	4.02 ± 0.12 ^g	12.11 ± 0.63 ^l	5.98 ± 0.18 ^{c,d}
4.40	2.32	–	51.40 ± 1.47 ^{d,e}	4.23 ± 0.15 ^f	16.49 ± 1.65 ⁱ	6.82 ± 0.87 ^{b,c}
6.60	2.32	–	22.50 ± 0.34 ^h	4.41 ± 0.24 ^{f,g}	20.98 ± 1.92 ^f	7.12 ± 0.91 ^b
8.80	2.32	–	20.40 ± 0.16 ^h	4.51 ± 0.13 ^{e,f}	17.26 ± 2.18 ^h	4.32 ± 0.15 ⁱ
1.10	4.64	–	20.80 ± 0.29 ^h	4.67 ± 0.18 ^{e,f}	7.54 ± 0.87 ^m	3.54 ± 0.39 ^j
2.20	4.64	–	24.90 ± 0.23 ^{g,h}	4.54 ± 0.12 ^{e,f}	15.32 ± 1.14 ^{i,j}	5.98 ± 0.66 ^c
3.30	4.64	–	41.30 ± 3.21 ^e	4.72 ± 0.91 ^e	20.76 ± 2.84 ^f	5.74 ± 0.43 ^c
4.40	4.64	–	83.20 ± 4.29 ^b	4.73 ± 0.43 ^e	28.43 ± 1.43 ^d	6.31 ± 0.25 ^c
6.60	4.64	–	27.70 ± 1.26 ^{f,g}	4.67 ± 0.67 ^{e,f}	23.12 ± 1.24 ^e	5.55 ± 0.12 ^d
8.80	4.64	–	23.80 ± 0.20 ^{g,h}	4.84 ± 0.72 ^e	16.39 ± 1.61 ⁱ	5.10 ± 0.87 ^{d,e}
1.10	2.32	1.4	21.50 ± 0.23 ^h	10.98 ± 0.82 ^a	6.20 ± 1.23 ^m	5.10 ± 1.17 ^{d,e}
2.20	2.32	1.4	26.70 ± 0.98 ^h	10.81 ± 0.18 ^a	14.87 ± 1.60 ^k	7.00 ± 1.11 ^b
3.30	2.32	1.4	29.90 ± 1.23 ^{f,g}	9.52 ± 0.34 ^{a,b}	18.32 ± 0.89 ^g	5.00 ± 0.56 ^f
4.40	2.32	1.4	52.30 ± 2.06 ^d	9.73 ± 0.41 ^{a,b}	23.22 ± 1.09 ^e	7.38 ± 0.10 ^b
6.60	2.32	1.4	75.30 ± 3.21 ^c	8.57 ± 0.44 ^b	26.34 ± 1.14 ^d	5.62 ± 0.18 ^d
8.80	2.32	1.4	22.00 ± 1.25 ^h	9.89 ± 0.33 ^{a,b}	15.45 ± 0.28 ^{i,j}	5.28 ± 0.67 ^{d,e}
1.10	4.64	1.4	29.60 ± 1.16 ^{f,g}	8.22 ± 0.33 ^b	20.10 ± 2.16 ^f	4.05 ± 0.50 ^h
2.20	4.64	1.4	34.70 ± 1.15 ^f	9.67 ± 0.13 ^{a,b}	26.20 ± 1.31 ^d	5.72 ± 0.61 ^c
3.30	4.64	1.4	87.40 ± 2.33 ^b	7.51 ± 0.20 ^c	37.76 ± 3.46 ^b	9.74 ± 0.64 ^a
4.40	4.64	1.4	113.98 ± 3.80 ^a	10.21 ± 0.57 ^a	43.56 ± 5.31 ^a	6.92 ± 0.69 ^{b,c}
6.60	4.64	1.4	59.90 ± 1.40 ^c	6.07 ± 0.30 ^{c,d}	31.20 ± 2.84 ^c	5.12 ± 0.67 ^{d,e}
8.80	4.64	1.4	33.70 ± 1.26 ^f	7.38 ± 0.41 ^c	22.45 ± 2.23 ^e	5.92 ± 0.32 ^c

Values represent mean ± SE of three repeated experiments each with ten replications. Means in a column with the different letter (superscript) are significantly different according to DMRT ($P < 0.05$)

Antioxidant activity

Free-radical scavenging activity (FRSA) was determined to evaluate the antioxidant potential of in vitro propagated plants and this was compared with level of wild-grown plants. Considering the fact that the mechanisms of antioxidant processes are complex, an approach with multiple assays in screening work is highly advisable (Sacchetti et al. 2005; Matkowski 2008; Amoo et al. 2012).

In the DPPH assay, the wild-grown plants of *S. corymbosa* showed higher radical scavenging activity compared with the positive standard (BHT) and in vitro propagated plants are presented in Table 6. To determine the relationship between the levels of the total phenolics and the antioxidant capacity of the extracts, we performed correlation and regression analysis. Total phenolic content of wild-grown plants' methanolic extract was correlated with radical scavenging activity against DPPH and flavonoids are presented in Table 7, whereas in in vitro propagated plants' methanolic extract, the

significant correlation was not observed. The radical scavenging activity of extracts can be credited to the presence of its major phenolic compounds (Guimaraes et al. 2010). The antioxidant activity of phenolic compounds is related to the hydroxyl groups linked to the aromatic ring, which are capable of donating hydrogen atoms with electrons and stabilizing free radicals (Dorman et al. 2003; Yanishlieva et al. 2006). In *P. glabrescens*, *C. pumilum* and *M. officinalis* higher antioxidant activity correlated with higher total phenolic contents (Garcia-Perez et al. 2012; Khateeb et al. 2012; Barros et al. 2013).

The Trolox equivalent antioxidant capacity (TEAC) was measured using the improved ABTS radical cation decolorization assay. The decolorization of ABTS⁺ cation radical is an unambiguous way to measure the total equivalent antioxidant capacity of test compounds or plant samples. TEAC is a measurement of the effective antioxidant activity of the extract. A higher TEAC value would imply greater antioxidant activity of the sample. This assay was calibrated with the water-soluble α -tocopherol

Table 4 Subcultures from in vitro derived nodal explant of *Sweetia corymbosa*

Growth regulator ($\mu\text{M}/\text{l}$)	Sub culture									
	I		II		III		VI		V	
	No. of shoots	Shoot length (cm)	No. of shoots	Shoot length (cm)	No. of shoots	Shoot length (cm)	No. of shoots	Shoot length (cm)	No. of shoots	Shoot length (cm)
1 4.40 μM (BA)	25.90 \pm 0.31	3.05 \pm 0.14	26.00 \pm 0.42	2.82 \pm 0.11	25.60 \pm 0.22	3.01 \pm 0.09	26.10 \pm 0.23	3.12 \pm 0.11	26.00 \pm 0.14	2.87 \pm 0.05
2 4.40 μM (BA) + 1.3 μM (NAA)	36.50 \pm 0.34	3.14 \pm 0.15	37.30 \pm 0.30	2.82 \pm 0.11	38.70 \pm 0.26	3.20 \pm 0.09	40.50 \pm 0.26	3.32 \pm 0.21	35.70 \pm 0.33	3.17 \pm 0.13
3 4.40 μM (BA) + 4.64 μM (Kn)	84.30 \pm 0.26	4.23 \pm .06	84.90 \pm 0.17	4.37 \pm .08	86.30 \pm 0.26	4.37 \pm 0.08	87.70 \pm 0.21	4.39 \pm 0.07	86.50 \pm 0.52	4.37 \pm 0.07
4 4.40 μM (BA) + 4.64 μM (Kn) + 1.4 μM (GA ₃)	112.01 \pm 0.12	10.19 \pm 0.92	113.87 \pm 0.87	10.12 \pm 0.81	117.71 \pm 0.11	9.67 \pm 0.77	121.98 \pm 0.56	10.67 \pm 0.54	112.17 \pm 0.89	11.43 \pm 0.98

Values represent mean \pm SE of three repeated experiments each with five replications

analogue, Trolox. Similarly to DPPH assay, the wild-grown plants showed the highest amount of ABTS⁺ radical quenching ability and then BHA and in vitro propagated plants material (Table 6). It is speculated that the activity may be contributed by the hydrogen-donating compounds that are most likely to be present in the polar solvents (Al-Zubairi et al. 2011). Due to their effective ABTS⁺ scavenging property they can be classified as agreeable antiradical agents, equally effective as Trolox. From the correlation analysis, it is evident that the phenolics and flavonoids in the methanolic extract of wild-grown plants were responsible for reducing activity (Table 7).

Antioxidants can be explained as reductants and inactivators of oxidants (Siddhuraju and Becker 2007). Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity. Antioxidative activity has been proposed to be related to reducing power. Therefore, the antioxidant activity of the methanolic extract of both wild-grown and in vitro regenerated plants were estimated for their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe(II). The FRAP activities of wild-grown plants of *S. corymbosa* and in vitro propagated plants are presented in Table 6. Similar to DPPH and ABTS radical scavenging activity, the reducing power of wild-grown plants was significantly higher than in vitro propagated plants. From the correlation analysis, it is evident that phenolics ($r^2 = 0.972$, $P < 0.01$) and flavonoids ($r^2 = 0.956$, $P < 0.05$) in the methanolic extract of wild-grown plants were the main contributors for their reducing activity (Table 7). FRAP assay was used by several authors for the assessment of antioxidant activity of medicinal plant samples (Thenmozhi et al. 2013; Sowndhararajan and Kang 2013).

The antioxidant activity (metal chelating) showed the same trend as the DPPH, ABTS and FRAP in the two types of plants (Table 6). The metal chelating activities of wild-grown plants and in vitro propagated plants' methanolic extracts of *S. corymbosa* were 127.93 \pm 10.05 mg EDTA/g extract and 65.32 \pm 3.14 mg EDTA/g extract, respectively. Phenolics ($r^2 = 0.997$, $P < 0.01$) and flavonoids in wild-grown plants' extracts ($r^2 = 0.990$, $P < 0.01$) were responsible for metal chelating activity. The differences in correlation coefficient among different antioxidant methods indicate the fact that single assay may not be used to assess the total antioxidant activity (Silva et al. 2006).

Antimicrobial activity

Antibacterial activity was compared between in vitro propagated and wild-grown plants. The results of antibacterial assay are presented in Table 8. The result from the

Table 5 The in vitro rooting of *S. corymbosa* microshoots in the presence of different auxins

IBA ($\mu\text{M/l}$)	NAA ($\mu\text{M/l}$)	IAA($\mu\text{M/l}$)	No of roots	Root length (cm)
2.4	–		7.90 ± 0.17^d	3.53 ± 0.23^b
4.9	–		10.80 ± 0.24^b	3.00 ± 0.00^c
9.8	–		14.40 ± 0.26^a	3.94 ± 0.19^a
–	2.6		3.40 ± 0.16^f	$2.72 \pm 0.20^{c,d}$
–	5.3		5.70 ± 0.15^e	2.40 ± 0.16^e
–	10.7		$8.20 \pm 0.24^{c,d}$	$2.30 \pm 0.12^{e,f}$
		2.8	5.70 ± 0.26^e	3.00 ± 0.00^c
		5.6	8.60 ± 0.16^c	2.00 ± 0.21^f
		11.2	11.10 ± 0.31^b	$2.50 \pm 0.19^{d,e}$

Values represent mean \pm SE of three repeated experiments each with ten replications. Means in a column with the different letter (super-script) are significantly different according to DMRT ($P < 0.05$)

disc diffusion method measured in inhibition zone (IZ in mm) of in vitro propagated and wild-grown plants methanol extracts against bacterial strains ranged from 2.50 to 11.50 mm (Table 8). Methanol extract of wild-grown plants of *S. corymbosa* showed high antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, *S. pneumoniae*, *P. aeruginosa*, and *K. pneumoniae* at 100 $\mu\text{g/ml}$. It is evident from Table 8 that the antibacterial effect of methanol extract was dose-dependent and a group of secondary compounds was effective in their antimicrobial properties. These results are also supported by estimation of total phenolic contents (TPC) and total flavonoid contents (TFC) in the extracts, where the wild-grown plants were found to possess greater amount of phenolic and total flavonoid

Table 6 Total phenolics (TPC) and flavonoids content (TFC) and antioxidant activity of in vitro regenerated plants and wild-grown plants' methanol extracts *S. corymbosa*

Sample (extract)	Total phenolics content (TPC) mg/g ^a	Total flavonoids content (TFC) mg/g ^b	DPPH IC ₅₀ ($\mu\text{g/ml}$)	FRAP mmol Fe(II)/g extract ^c	ABTS μmol trolox/g extract ^d	Metal chelating activity mg EDTA/g extract ^e
Wild grown plants	63.38 ± 2.38	146.57 ± 1.68	22.91^a	1955.29 ± 35.14^a	32711.05 ± 339.94^a	127.93 ± 10.05^a
In -vitro propagated plants	34.16 ± 1.86	69.88 ± 2.42	51.12^c	0913.98 ± 45.67^c	19243.54 ± 309.21^c	65.32 ± 3.14^b
BHA			34.59^b	1213.89 ± 80.86^b	21682.08 ± 359.47^b	62.37 ± 4.68^b

Values are mean of triplicate determination ($n = 3$) \pm standard deviation. Mean values followed by different superscript in a column are significantly different ($P < 0.05$)

BHA Butylated hydroxyl anisole

^a Gallic acid equivalents (GAE)

^b Rutin equivalent (RE)

^c mmol of ferrous equivalents/g extract

^d μmol of trolox equivalents/g extract

^e mg of EDTA equivalent/g extract

Table 7 Correlation between phenolics, flavonoids, and different antioxidant parameters of in vitro regenerated plants and wild-grown plants' methanol extract of *S. corymbosa*

Parameters	Phenolics		Flavonoids	
	Wild-grown plants extract	In vitro propagated plants extract	Wild-grown plants extract	In vitro propagated plants extract
DPPH	-0.990^{**}	0.268	-0.972^{**}	0.599
ABTS	0.962^{**}	0.988^{**}	0.967^*	0.369
FRAP	0.972^{**}	-0.672	0.956^*	0.960^{**}
MCA	0.997^{**}	0.982^{**}	0.990^{**}	0.616

FRAP ferric reducing antioxidant power assay, MCA metal chelating activity

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed)

content than in vitro propagated plants. The differences between the responses of wild-grown plants and in vitro propagated plants could be attributed to the differences in the chemical constituents of the plant cells (phenolic and flavonoid contents) that are likely to develop in response to the surrounding environmental conditions (Parseimehr et al. 2010; Mohanty et al. 2011; Khateeb et al. 2012; Ahmad et al. 2013; Baskaran et al. 2013).

HPLC–ESI-MS analysis

The phytochemicals present in most *Swertia* species have been found to be iridoids, xanthenes, mangiferin and C-glucoflavones. Xanthenes are the major class of compounds among the chemical constituents of this genus.

Table 8 Antimicrobial activity of in vitro regenerated plants and wild-grown plants' methanol extract of *S. corymbosa*

Bacteria	Zone of inhibition (mm)								
	Wild-grown plants extract				In vitro propagated plants extract				Gentamicin 10 µg/disc
	25 µg	50 µg	75 µg	100 µg	25 µg	50 µg	75 µg	100 µg	
<i>E. coli</i> (ATCC 25922)	3.00	4.50	5.50	7.00	–	–	2.50	3.50	11.00
<i>P. aeruginosa</i> (ATCC 27853)	–	–	2.50	3.50	–	–	–	2.00	9.50
<i>S. aureus</i> (ATCC 29213)	3.00	5.50	7.50	9.00	–	2.50	5.00	5.50	12.00
<i>S. pneumoniae</i> (ATCC 33400)	2.41	3.50	4.00	5.00	–	–	–	3.50	8.50
<i>K. pneumoniae</i> (ATCC 10031)	2.50	3.00	3.50	4.50	–	–	3.50	4.00	13.50
<i>B. subtilis</i> (ATCC 6633)	4.50	5.50	8.00	11.50	3.50	4.00	5.00	7.50	17.00

The results are expressed as the means of three repeat experiments

Xanthenes are yellow pigments characteristic of some higher plant families, including *Gentianaceae* and *Guttiferaceae* (Hostettmann and Hostettmann 1989). The growing interest in xanthenes is based on their diverse biological activities, which include hepatoprotective, anti-inflammatory, anti-tumour activity and antidiabetic activity (Pinto et al. 2005; Muruganandan et al. 2005; Tian et al. 2010). The iridoids (mainly secoiridoid glucosides) appear to be present in all species investigated with a predominance of swertiamarin, sweroside and gentiopicroside. The distribution of iridoids has been shown to have considerable value as a systematic character since they occur almost exclusively in *Swertia* species. According to the HPLC condition mentioned earlier, the chromatograms of both wild-grown plants and in vitro propagated plants' methanolic extracts of *S. corymbosa* are presented in Figs. 2a, b and 3. Ten compounds were identified as the major components of these extracts. In the MS spectra, the retention times and mass fragmentation of the ten compounds were shown in Table 9.

The accumulation of secondary metabolites is affected by the degree of cellular differentiation and organization of the tissue (Verpoorte and Memelink 2002). The bioactive compound content was thus varied and tissue dependent (Baskaran and Jayabalan 2008; Lystvan et al. 2010). The concentrations of certain secondary products are strongly dependent on the growth conditions and type of stress has a major impact on the synthesis and accumulation of these compounds (Bohnert et al. 1995). They reviewed many investigations and concluded that plants that are exposed to drought stress produce a greater amount of secondary products. The employment of stress factors, such as osmotic shock, is an important strategy for the improvement of the secondary metabolite production in plant cell cultures (Zhao et al. 2001). The present study revealed that secondary metabolite content was higher in wild-grown plants than in vitro propagated plants' extract of *S. corymbosa* (Table 9). In vitro cultured seem to be under less

stress, producing lower amounts of phenolic compounds (secondary metabolites) and their accumulation. In fact, in vitro cultured samples were grown under controlled light, temperature and nutrients. *Gentianaceae* family consists of the three secoiridoid-glycosides: gentiopicroside, swertiamarin, and sweroside are the main characteristic compounds (Szucs et al. 2002; Carnat et al. 2005). Similarly in *S. corymbosa* gentiopicroside (40.726 mg/g), swertiamarin (12.987 mg/g), sweroside (9.092 mg/g) and a xanthone swertianin (29.598 mg/g) were found to be the main chemical compounds. Thus, swertiamarin and isovitexin present in the plants derived from wild were missing in the shoots of in vitro cultured plants (Table 9). Similar phenomenon was also observed in the hairy roots of *G. punctata* (Menkovic et al. 2000) and in vitro shoot cultured plants of *Gentiana dinarica* (Branka et al. 2013). In the present investigation, gentiopicroside, swertianin, and swertiamarin are higher in the plants derived from nature than in tissue cultures, as reported in *Gentiana manshurica* (Zhiguo et al. 1993) and *Gentiana macrophylla* (Zhang et al. 2010; Wu et al. 2011).

In conclusion, the present study is the first to report using nodal explants the successful plant regeneration in *S. corymbosa* (Griseb.) Wight ex C.B. Clarke. The protocol described here could be applied in a propagation program for genetic resource conservation and commercial purposes. The total phenolics, flavonoid content and antioxidant activity of in vitro propagated plants methanol extract were lower than that of wild-grown plants' methanol extract. In addition, we provide evidence that anti-bacterial activity of *S. corymbosa* extracts revealed that wild-grown plants' methanol extract at 100 µg/ml exhibited strong antibacterial activity against *S. aureus*, *B. subtilis*, *E.coli*. Studies presented in this paper provide a clear picture of secondary metabolite composition of *S. corymbosa* plants and tissues grown under conditions of in vitro culture. Although the secoiridoids and xanthenes were present both in plants from nature and those in in vitro propagated

Fig. 2 HPLC chromatograms of the metabolites. **a** Wild-grown plants' methanolic extract of *Swertia corymbosa*. **b** in vitro propagated plants' methanolic extract *Swertia corymbosa*

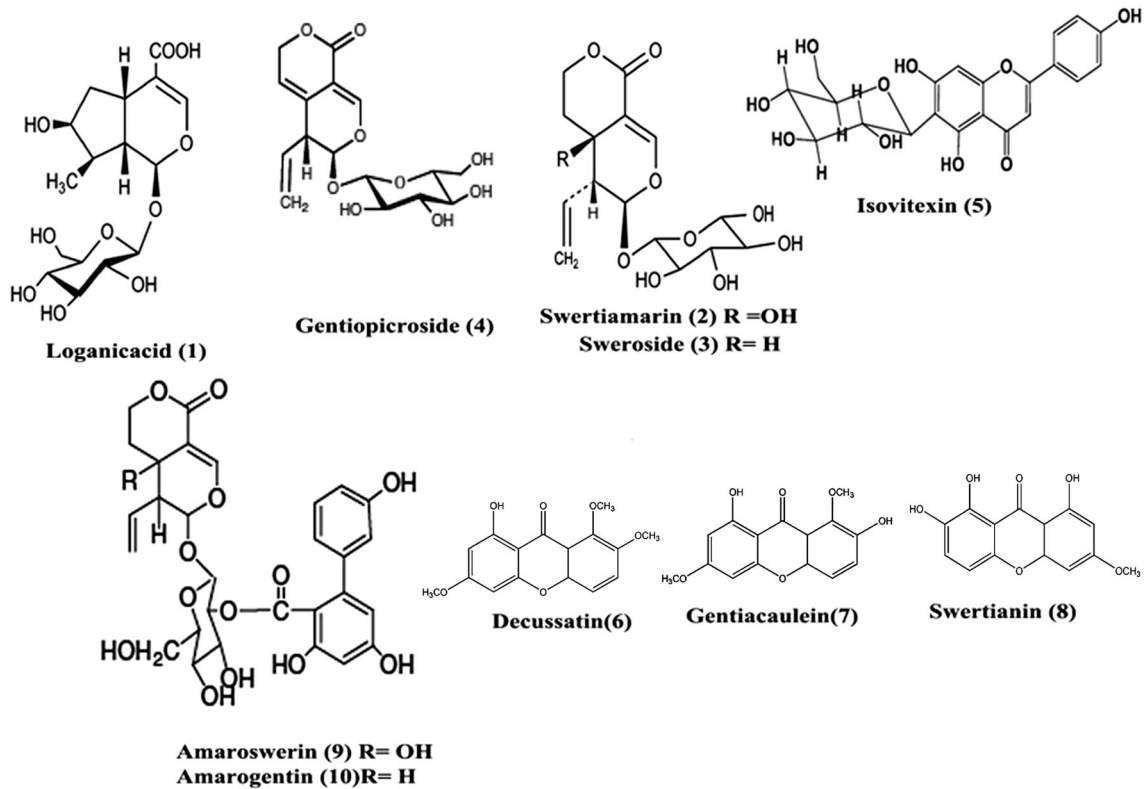
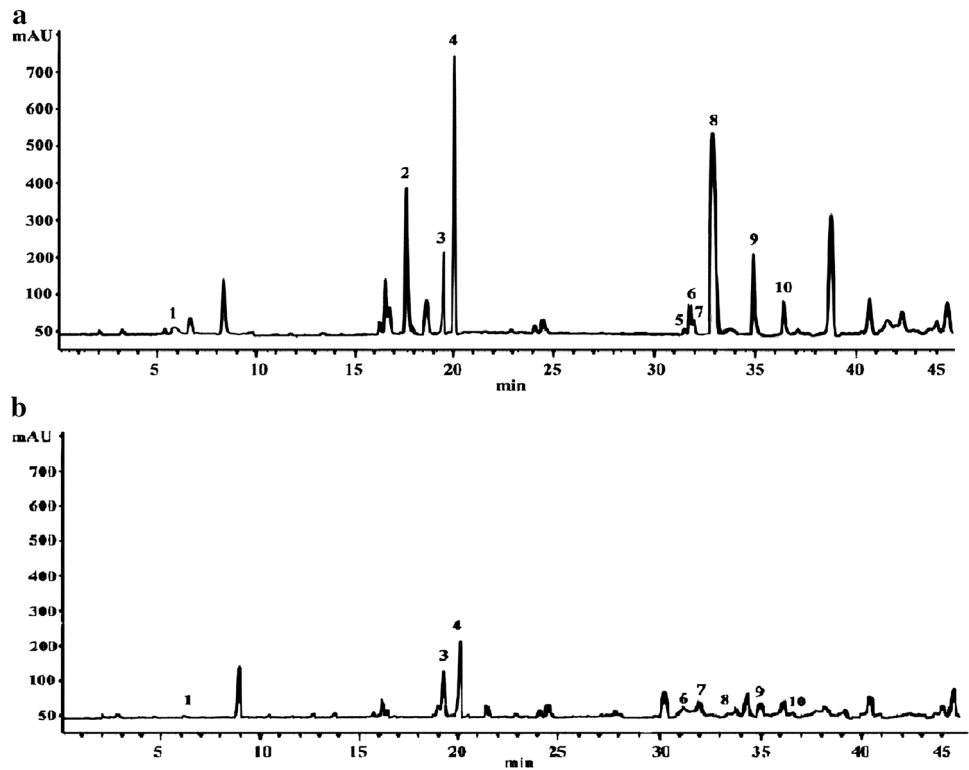


Fig. 3 Chemical structures of the investigated compounds

Table 9 Characterization and quantification of compounds in methanolic extracts of *Swertia corymbosa* by HPLC/ESI-MS/UV data

S. No.	Compounds	t_R	Mass	Product ions (m/z-relative abundance, %)	UV λ_{max} (nm)	Secondary metabolite content mg/gDW	
						Wild-grown plants extract	In vitro propagated plants extract
1	Loganic acid	5.7	394	394 (10), 377 (15), 259 (30), 341 (100), 298 (100), 279 (10), 260 (21)	256, 282, 338	0.569	0.182
2	Swertiamarin	17.3	375	397 (50), 235 (40), 217 (54), 165 (100).	238	12.987	–
3	Sweroside	19.4	359	359 (20), 341 (32), 311 (40), 197 (100), 127 (30)	245, 265	9.092	3.210
4	Gentiopicroside	20.0	356	357 (20), 197 (100), 127 (60)	273, 257, 219,	40.726	8.536
5	Isovitexin	31.8	433	433 (45), 415 (60), 397 (64), 379(45),360 (20), 367 (60)	214, 279, 344	0.531	–
6	Gentiauclein	31.9	288	288 (62), 270 (125), 245 (62), 229 (16), 202 (20)	238, 261, 312, 376	1.133	0.485
7	Decussatin	32.2	302	302 (77), 287 (125), 273 (10), 259 (50), 201 (11)	234, 254, 312, 372	0.935	1.014
8	Swertianin	32.8	274	274 (125), 246 (61), 245 (96), 231 (60), 202 (30), 185 (20)	234, 252, 315, 374	29.598	0.058
9	Amaroswertin	35.0	602	602 (15), 413 (13), 397 (70), 255 (20), 251 (20), 235 (30), 185 (25), 165 (30)	225, 270, 310	13.479	1.121
10	Amorogentin	36.3	587	587 (25), 391 (50), 229 (100), 197 (30), 127 (40)	225, 270, 310	6.750	1.148

The secondary metabolites were measured from three independent replicates and repeated thrice

plants, their levels in wild-grown and in vitro propagated plants showed significant quantitative differences. In future, we suggest evaluating the role of abiotic and biotic stresses at in vitro level to improve these phytochemicals.

Author contribution G. Mahendran designed, performed the experiment work and wrote the manuscript. V. Narmatha Bai helped with co-ordination of tissue culture and interpretation of data.

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