

## RESEARCH NOTE



# Bioreactor type affects the accumulation of phenolic acids and flavonoids in microshoot cultures of *Schisandra chinensis* (Turcz.) Baill.

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## Abstract

Microshoots of the East Asian medicinal plant species *Schisandra chinensis* (Chinese magnolia vine) were grown in bioreactors characterized by different construction and cultivation mode. The tested systems included two continuous immersion systems—a cone-type bioreactor (CNB) and a cylindrical tube bioreactor (CTB), a nutrient sprinkle bioreactor (NSB), and two temporary immersion systems (TIS)—RITA® and Plantform. Microshoots were grown for 30 and 60 days in the MS medium enriched with 1 mg l<sup>-1</sup> NAA and 3 mg l<sup>-1</sup> BA. The accumulation of two groups of phenolic compounds: phenolic acids and flavonoids in the bioreactor-grown *S. chinensis* biomass, was evaluated for the first time. In the microshoot extracts, seven phenolic acids: chlorogenic, gallic, p-hydroxybenzoic, protocatechuic, syringic, salicylic and vanillic, and three flavonoids: kaempferol, quercitrin and rutoside, were identified. The highest total amount of phenolic acids (46.68 mg 100 g<sup>-1</sup> DW) was recorded in the biomass maintained in the CNB for 30 days. The highest total content of flavonoids (29.02 mg 100 g<sup>-1</sup> DW) was found in the microshoots maintained in the NSB for 30 days. The predominant metabolites in all the tested systems were: gallic acid (up to 10.01 mg 100 g<sup>-1</sup> DW), protocatechuic acid (maximal concentration 16.30 mg 100 g<sup>-1</sup> DW), and quercitrin (highest content 21.00 mg 100 g<sup>-1</sup> DW).

## Key message

The influence of bioreactor type on the accumulation of phenolic acids and flavonoids in microshoot cultures of *Schisandra chinensis* was proven and optimized.

**Keywords** Plant-dedicated bioreactors · Chinese magnolia vine · Schizandra · Phenolic secondary metabolites · Protocatechuic acid · Quercitrin

## Abbreviations

ADB	Accumulated dry biomass
BA	6-Benzyladenine
CNB	Cone-type bioreactor
CTB	Cylindric tube bioreactor

DW	Dry weight
FW	Fresh weight
Gi	Growth index
HPLC–DAD	High-performance liquid chromatography coupled with diode-array detection
NAA	1-Naphthaleneacetic acid
μ	Specific growth rate
MS	Murashige and Skoog
NSB	Nutrient sprinkle bioreactor
TCM	Traditional Chinese medicine
TIS	Temporary immersion systems

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Research on secondary metabolites production in bioreactor-grown biomasses, constitute an important topic in plant

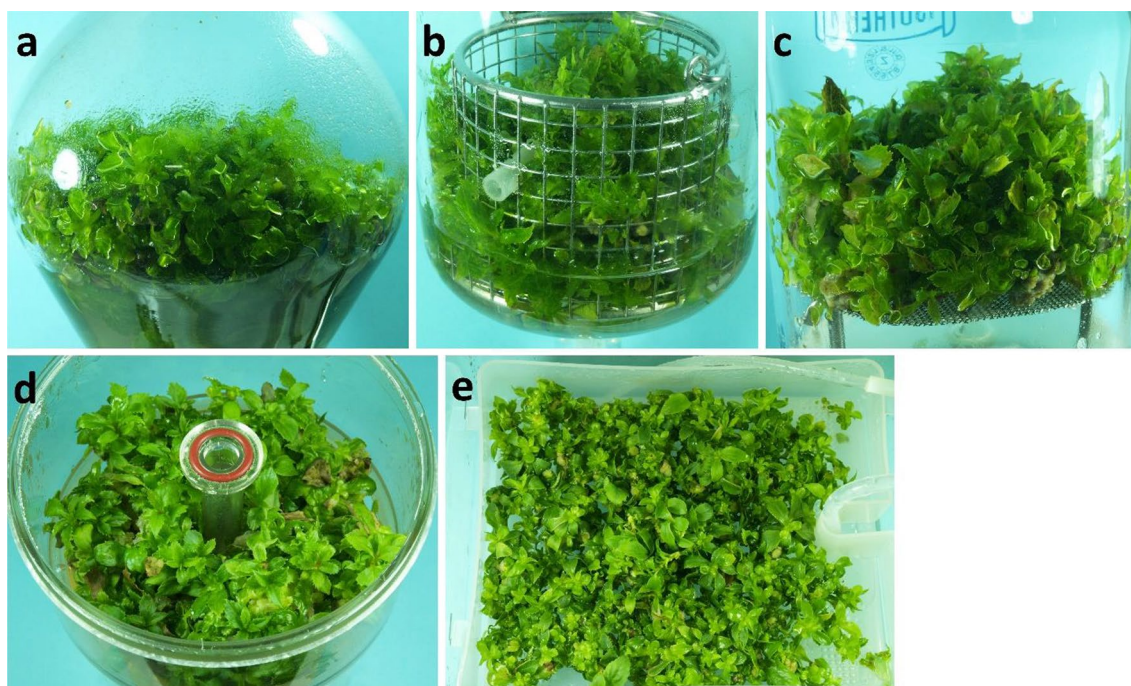
biotechnology. The results of such studies are important from a practical point of view and the prospects of producing valuable, biologically active metabolites in such systems continue to draw scientists' attention (Verpoorte et al. 2002; Karuppusamy 2009). The latest studies have demonstrated the possibility of large-scale production of metabolites like: artemisinin (antimalarial drug) in cultures of *Artemisia annua* grown in a specially-designed stirred tank bioreactor, plumbagin (anticancer compound) in *Plumbago rosea* cultures maintained in a customized reaction kettle (Jose et al. 2016), and steviol glycosides (low-calorie glucoside sweeteners) in *Stevia rebaudiana* cultures grown in a temporary immersion bioreactor (Vives et al. 2017). In all studies involving large-scale in vitro systems, the selection of proper bioreactor type and optimization of process parameters is crucial for maximizing secondary metabolite production.

The role of plant-derived phenolic substances in diverse areas such as medicine, cosmetology and food industry, is invaluable. Secondary metabolites like flavonoids and phenolic acids exhibit many important, scientifically proven, biological activities, e.g. antioxidant (Young and Woodside 2001; Krishnaiah et al. 2011), anti-inflammatory (Zhang and Tsao 2016), or anticancer (Roleira et al. 2015). These compounds are used either in pure form or as constituents of herbs, plant extracts and functional foods.

In the current study, highly differentiated microshoot cultures of rare, East Asian plant species—*Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine) were

investigated as a source of flavonoids and phenolic acids. *S. chinensis* fruits constitute raw pharmacopoeial material and are especially well-known in the traditional medicine of East-Asian countries. However, they have also been included in current phytotherapy all over the world (Barnes and Anderson 2007; World Health Organization 2007; European Directorate for the Quality of Medicines 2017; Szopa et al. 2017a). Their medicinal properties, e.g. hepatoprotective, immunostimulant, adaptogenic and anti-cancer, are determined mainly by the presence of dibenzocyclooctadiene type lignans (aka “schisandra lignans”), a *Schisandra* genus-specific group of secondary metabolites (Opletal et al. 2004; Szopa et al. 2017a). However, recent studies have demonstrated that phenolics present in *S. chinensis* act synergistically with lignans, thus serving as co-adjuvants and increasing biological activities of the plant (Szopa and Ekiert 2012; Cheng et al. 2013; Mocan et al. 2014). The compounds of particular interest are phenolic acids and flavonoids which themselves exhibit beneficial biological effects.

In this study, in vitro microshoot cultures of *S. chinensis* were evaluated for the production of phenolic compounds in five bioreactors designed for differentiated plant in vitro cultures (systems nomenclature after Kim et al. 2004; Steingroewer et al. 2013). Of these, three were custom-made systems: a cone-type balloon bioreactor (CNB) a cylindrical tube bioreactor (CTB) with internal rack for biomass immobilization and a nutrient sprinkle bioreactor (NSB), and two



**Fig. 1** Microshoot cultures of *Schisandra chinensis* grown in different bioreactor systems: **a** cone-type bioreactor (CNB); **b** cylindrical tube bioreactor (CTB); **c** nutrient sprinkle bioreactor (NSB); **d** RITA® bioreactor (open lid); **e** Platform bioreactor (open lid)

were commercially available, temporary immersion bioreactors—RITA® (Vitropic, France) and Plantform (PlantForm, Sweden) (Fig. 1). The construction details and setup of the bioreactors had been described by us previously (Szopa et al. 2017c). To our knowledge, this is the first work aimed at optimizing conditions for favourable biomass growth and phenolic acids and flavonoids production in bioreactor-grown *S. chinensis* microshoots.

As described previously, the microshoots were cultivated in the Murashige & Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 1.0 mg l<sup>-1</sup> 1-naphthalene acetic acid (NAA) and 3.0 mg l<sup>-1</sup> 6-benzyladenine (BA), which had previously been demonstrated to provide the best biomass growth and secondary metabolites production in *S. chinensis* in vitro cultures (Szopa and Ekiert 2013, 2015; Szopa et al. 2016). All bioreactor systems were inoculated at 3/100 w/v ratio which corresponds to 15 g of microshoots per 500 ml of medium in CNB, CTB, NSB and Plantform bioreactors, and 6 g of microshoots per 200 ml of medium for RITA® container. The cultures were grown in constant artificial light (Philips fluorescent white lamps, 90 ± 2 μmol m<sup>-2</sup> s<sup>-1</sup>), at 24 ± 2 °C. The bioreactor cultures were maintained for 30 and 60 days and the collected biomasses were evaluated for growth parameters including fresh (FW) and dry (DW) weight, growth index (Gi), accumulated dry biomass (ADB) and specific growth rate (μ). The recorder values and formulae used are presented in Table 1. Afterwards, the microshoots and corresponding media samples (40 ml) were freeze dried (Lyovac GT2, Finland) and subjected to

phytochemical analysis. The bioreactor experiments were done in four repetitions.

The lyophilized and pulverized biomass samples (0.5 g from each bioreactor, four samples for each bioreactor type) were extracted with methanol (50 ml) for 2 h under reflux condenser (Harborne 1984). The extracts were transferred to crystallizers through a filter paper (Whatman paper) and left at room temperature to evaporate the solvent. The dry residue was dissolved in 2 ml of methanol (HPLC grade purity, Merck) and subjected to HPLC analysis.

Qualitative and quantitative HPLC–DAD analyses were performed according to the previously published protocols (Ellnain-Wojtaszek and Zgorzka 1999; Sułkowska-Ziaja et al. 2017; Szopa et al. 2017b). The equipment used and method parameters were as follows: HPLC–DAD system (Merck-Hitachi), Purospher Merck analytical RP-18 column (4 × 250 mm, 5 μm), mobile phase (gradient program): A—methanol with 0.5% acetic acid (1:4 v/v); B—methanol, flow—1 ml min<sup>-1</sup>, injection—10 μl, λ—254 nm, temp. 25 °C. The compounds were identified by co-chromatography with reference substances and comparison of their retention times and quantified using calibration curves. The phenolic acid standards (including benzoic and cinnamic acid and their derivatives) were as follows (t<sub>r</sub> values in minutes): gallic (3.86), neochlorogenic (4.98), protocatechuic (5.91), 3,4-dihydroxyphenylacetic (6.93), gentisic (10.00), chlorogenic (11.20), p-hydroxybenzoic (12.53), hydrocaffeic (15.37), vanillic (16.44), caffeic

**Table 1** Growth parameters of *Schisandra chinensis* microshoots maintained for 30 and 60 days in different bioreactor systems

Bioreactor type	Growth period (days)	FW (g l <sup>-1</sup> )	DW (g l <sup>-1</sup> )	Gi <sup>a</sup>	ADB (g l <sup>-1</sup> ) <sup>b</sup>	μ (d <sup>-1</sup> ) <sup>c</sup>	Biomass productivity (g l <sup>-1</sup> d <sup>-1</sup> )
CNB	30	120.29 ± 17.15	6.88 ± 0.93	3.01 ± 0.57	4.68 ± 0.93	0.0377 ± 0.0048	0.229 ± 0.031
	60	266.99 ± 25.55	15.41 ± 0.88	7.90 ± 0.85	13.21 ± 0.88	0.0324 ± 0.0010	0.257 ± 0.015
CTB	30	128.09 ± 22.80	8.08 ± 0.94	3.22 ± 0.75	5.87 ± 0.94	0.0431 ± 0.0040	0.269 ± 0.031
	60	233.91 ± 13.30	14.63 ± 0.71	6.73 ± 0.47	12.43 ± 0.71	0.0315 ± 0.0008	0.244 ± 0.012
NSB	30	126.34 ± 20.30	7.76 ± 1.00	3.21 ± 0.68	5.56 ± 1.00	0.0417 ± 0.0043	0.259 ± 0.033
	60	274.01 ± 21.13	15.50 ± 0.43	8.13 ± 0.70	13.29 ± 0.43	0.0325 ± 0.0005	0.258 ± 0.007
RITA®	30	134.62 ± 5.84	7.49 ± 0.21	3.49 ± 0.19	5.28 ± 0.21	0.0408 ± 0.0009	0.250 ± 0.007
	60	353.37 ± 20.04	17.86 ± 0.72	10.78 ± 0.67	15.65 ± 0.72	0.0349 ± 0.0007	0.298 ± 0.012
Plantform	30	105.78 ± 3.68	6.23 ± 0.18	2.53 ± 0.12	4.02 ± 0.18	0.0346 ± 0.0010	0.208 ± 0.006
	60	294.19 ± 19.30	17.07 ± 1.14	8.81 ± 0.64	14.86 ± 1.14	0.0341 ± 0.0011	0.284 ± 0.019

Growth parameters of *Schisandra chinensis* microshoots maintained for 30 and 60 days in different bioreactor systems: FW fresh weight; DW dry weight; Gi growth index; ADB accumulated dry biomass; μ specific growth rate and biomass productivity

<sup>a</sup>According to (Grzegorzczuk and Wysokińska 2008):  $Gi = [(Fw_1 - Fw_0) / Fw_1]$ , where Gi is the growth index, Fw<sub>1</sub> is the weight of microshoots at the end of experiment and Fw<sub>0</sub> is the fresh weight of the inoculum

<sup>b</sup>According to (Pavlov et al. 2007):  $ADB = FDB - IDB$  where ADB is the accumulated dry biomass (in g/L), FDB is the final dry biomass (g/L) and IDB is the initial dry biomass (g/L)

<sup>c</sup>According to (Homova et al. 2010):  $\mu = \ln(X/X_0) / \Delta t$  where μ is the specific growth rate (1/d), X<sub>0</sub> and X are the initial and final biomasses (g/l) and Δt is the culture time interval (d)

(17.83), syringic (20.14), ferulic (30.28), salicylic (31.76), p-coumaric (34.31), sinapic (35.41), o-coumaric (36.62), m-coumaric (37.81), isoferulic (39.18) and rosmarinic (44.32) acids (Sigma-Aldrich, USA). Standards used to estimate flavonoids included ( $t_r$  values in minutes): vitexin (42.07), cynaroside (43.57), hyperoside (46.04), myricetin (48.01), rutoside (49.26), quercitrin (50.29), apigetrin (53.27), trifolin (54.47), quercetin (58.32), luteolin (60.97) and kaempferol (64.42) (Sigma-Aldrich, USA). The results were statistically analyzed with one-way ANOVA and the Tukey's range test (Statistica 12, Poland).

In all the types of bioreactors employed, *S. chinensis* microshoots showed vigorous growth, and no necrosis or medium browning was observed (Fig. 1). After the biomasses were collected, their growth parameters were determined (Table 1). The Gi parameter ranged from 2.53 to 3.49 and from 6.73 to 10.78 for microshoots cultivated for 30 and 60 days, respectively. Of the custom made systems, the NSB yielded the highest ADB after 60 days of cultivation. In terms of Gi, ADB and biomass productivity, the TIS bioreactors performed substantially better than other systems employed. The highest biomass increments were obtained with RITA<sup>®</sup> bioreactors which was reflected by high values of all growth parameters, most notably ADB and biomass productivity (Table 1).

Based on HPLC-DAD quantitative analyses, the total contents of phenolic acids varied from 19.16 mg 100 g<sup>-1</sup> DW (CTB, 60-day growth period) to 46.68 mg 100 g<sup>-1</sup> DW (CNB, 30-day growth period). Comparing the amounts of phenolic acids, we found that the highest total quantities were obtained in biomass of the cultures grown over 30-day growth periods in all the tested types of bioreactor. The maximal total amount of phenolic acids achieved in biomass extracts from the CNB bioreactor was, respectively, 1.50, 1.18, 1.43 and 1.35 times higher than in the CTB, NSB, RITA<sup>®</sup> and Plantform bioreactors (Table 2).

Out of the nineteen phenolic acids, the analyses confirmed the presence of seven compounds: chlorogenic, gallic, p-hydroxybenzoic, protocatechuic, syringic, salicylic and vanillic acids (Table 2). The quantities of individual phenolic acids ranged from 0.72 mg 100 g<sup>-1</sup> DW for syringic acid (CTB, 60-day growth period) to 16.30 mg 100 g<sup>-1</sup> DW for protocatechuic acid (CNB, 30-day growth period). Besides protocatechuic acid, a considerable amounts of gallic acid (maximal 10.01 mg 100 g<sup>-1</sup> DW, CNB, 30-day growth period) and salicylic acid (maximal 6.74 mg 100 g<sup>-1</sup> DW, SB, 30-day growth period) were also confirmed (Table 2).

The total amounts of estimated flavonoids ranged from 12.90 mg 100 g<sup>-1</sup> DW (CTB, 60-day growth period) to 29.02 mg 100 g<sup>-1</sup> DW (NSB, 30-day growth period). Comparing the amounts of flavonoids in the respective systems, we found that the highest total contents were obtained in microshoots grown over 30-day growth periods in all the

tested types of bioreactor. The maximal total amount of flavonoids achieved in biomass extracts from the NSB bioreactor was, respectively, 1.40, 1.32, 1.36 and 1.42 times higher than in the CNB, CTB, RITA<sup>®</sup> and Plantform bioreactors (Table 3).

From the eleven flavonoids estimated, three compounds were found: kaempferol, quercitrin and rutoside (Table 3). The quantities of kaempferol ranged from 0.18 mg 100 g<sup>-1</sup> DW (CTB, 60-day growth period) to 6.49 mg 100 g<sup>-1</sup> DW (SB, 30-day growth period), and rutoside from 0.88 mg 100 g<sup>-1</sup> DW (CTB, 60-day growth period) to 1.87 mg 100 g<sup>-1</sup> DW (Plantform, 30-day growth period). Quercitrin was the main metabolite; its amounts ranged from 11.85 mg 100 g<sup>-1</sup> DW (CTB, 60-day growth period) up to 21.00 mg 100 g<sup>-1</sup> DW (NSB, 30-day growth period) (Table 3).

In all experiments, none of the estimated phenols were found in the media samples, indicating that the investigated compounds were stored solely intracellularly.

Our study proved that biomass growth and secondary metabolite contents in *S. chinensis* microshoots depended on the type of bioreactor used and its mode of operation, including medium circulation and aeration, which is in agreement with previous reports (Liu et al. 2003; Zobayed et al. 2004; Paek et al. 2005), including our work on the production of dibenzocyclooctadiene type lignans in bioreactor-grown *S. chinensis* microshoots (Szopa et al. 2017c). In that study, the best conditions were provided by the Plantform bioreactor, in which, during a 30-day growth period, microshoots had accumulated the highest amounts of lignans (546.98 mg 100 g<sup>-1</sup> DW), with gomisins A, deoxyschisandrin and schisandrin as the major constituents (67.86, 77.66 and 118.59 mg 100 g<sup>-1</sup> DW, respectively) (Szopa et al. 2017c). As mentioned before, the pharmacological action of *S. chinensis* is the result of synergistic action of all the plant's components; in this case, phenolic compounds seem to play a major role besides lignans (Mocan et al. 2014; Szopa et al. 2017b). We had proved before that different types of in vitro cultures of *S. chinensis* were able to produce phenolic acids and flavonoids, and that their yield depended on the in vitro conditions used, such as the basal media formulation, concentration of phytohormones, duration of the growth period, lighting conditions, as well as the mode of cultivation (agar, agitated, stationary liquid) (Szopa and Ekiert 2012, 2016; Szopa et al. 2017b).

Under the current study, we made the first step towards scaling-up the production of phenolic compounds using *S. chinensis* microshoot cultures. Of the tested systems, the RITA<sup>®</sup> TIS bioreactor provided the highest biomass yields (Table 1). However, the total phenolic acids content was considerably greater in the CNB (Table 2), while that of flavonoids in the NSB (Table 3). In the CNB system, aeration-induced mechanical stress caused by tissue-medium contact resulted in lower biomass growth (Gi equal to 3.01

**Table 2** Phenolic acid contents (mg 100 g<sup>-1</sup> DW ± SD) in extracts from biomasses of *Schisandra chinensis* microshoots cultivated in different types of bioreactors (n = 4, p < 0.05)

Phenolic acids	Bioreactor type									
	CNB		CTB		NSB		RITA®		Plantform	
	Growth period (days)									
	30	60	30	60	30	60	30	60	30	60
Chlorogenic acid	4.65 ± 0.3 <sup>cde</sup> ghij	4.23 ± 0.14 <sup>cde</sup> efghij	2.46 ± 0.11 <sup>ab</sup> ef	2.40 ± 0.17 <sup>ab</sup> ef	3.75 ± 0.29 <sup>abc</sup> efghij	3.56 ± 0.88 <sup>abc</sup> efghij	2.32 ± 0.3 <sup>1</sup> abef	2.14 ± 0.98 <sup>ab</sup> ef	2.98 ± 0.27 <sup>ab</sup> ef	2.81 ± 0.45 <sup>gh</sup> ef
Gallie acid	10.01 ± 1.04 <sup>ab</sup> hij	6.67 ± 0.30 <sup>abc</sup> efghij	8.50 ± 0.52 <sup>bc</sup> efhij	2.75 ± 0.13 <sup>abc</sup> efgi	8.87 ± 0.35 <sup>bc</sup> efhij	3.64 ± 0.13 <sup>abc</sup> efghij	8.77 ± 0.62 <sup>bc</sup> efhij	3.25 ± 0.14 <sup>abc</sup> efghij	7.45 ± 0.32 <sup>ac</sup> efhij	2.44 ± 0.27 <sup>abc</sup> efghi
p-Hydroxybenzoic acid	3.58 ± 0.46 <sup>bc</sup> efghij	2.56 ± 0.08 <sup>abc</sup> de	1.82 ± 0.09 <sup>abc</sup> ef	1.74 ± 0.08 <sup>abc</sup> efgi	3.12 ± 0.28 <sup>cd</sup> efghij	3.11 ± 0.37 <sup>bc</sup> efghij	2.23 ± 0.54 <sup>abc</sup> ef	1.79 ± 0.41 <sup>abc</sup> efgi	2.18 ± 0.22 <sup>ac</sup> ef	2.08 ± 0.38 <sup>ac</sup> ef
Protocatechuic acid	16.30 ± 1.15 <sup>cd</sup> efghij	15.97 ± 0.66 <sup>cd</sup> efghij	8.38 ± 0.23 <sup>abc</sup> ef	5.07 ± 0.12 <sup>abc</sup> efghij	12.20 ± 0.47 <sup>abc</sup> efghij	9.33 ± 0.63 <sup>abc</sup> ef	9.87 ± 0.84 <sup>abc</sup> ef	8.52 ± 0.68 <sup>abc</sup> efghij	10.85 ± 0.58 <sup>abc</sup> ef	9.01 ± 0.42 <sup>abc</sup> ef
Salicylic acid	5.66 ± 0.06 <sup>de</sup> ij	5.91 ± 0.19 <sup>de</sup> ij	5.09 ± 0.11 <sup>cd</sup> ef	5.00 ± 0.09 <sup>abc</sup> ef	6.74 ± 0.18 <sup>abc</sup> efghij	6.05 ± 0.86 <sup>abc</sup> efghij	5.27 ± 0.25 <sup>cd</sup> ef	4.87 ± 0.36 <sup>abc</sup> ef	5.84 ± 0.36 <sup>cd</sup> efghij	4.29 ± 0.40 <sup>bc</sup> efghij
Syringic acid	0.77 ± 0.03 <sup>ef</sup> ghij	0.74 ± 0.02 <sup>ef</sup> ghij	1.92 ± 0.06 <sup>abc</sup> defghij	0.72 ± 0.02 <sup>ef</sup> ghij	0.84 ± 0.11 <sup>ef</sup> ghij	0.83 ± 0.17 <sup>ef</sup> ghij	0.98 ± 0.22 <sup>abc</sup> defhij	0.74 ± 0.11 <sup>ef</sup> ghij	1.25 ± 0.11 <sup>abc</sup> defghij	0.87 ± 0.09 <sup>bc</sup> efghij
Vanillic acid	5.71 ± 0.50 <sup>bc</sup> defghij	3.85 ± 0.28 <sup>abc</sup> defghij	2.92 ± 0.05 <sup>abc</sup> defhij	1.50 ± 0.04 <sup>abc</sup> defghij	4.03 ± 0.27 <sup>cd</sup> efghij	3.91 ± 0.13 <sup>cd</sup> efghij	3.21 ± 0.21 <sup>abc</sup> defhij	1.86 ± 0.54 <sup>abc</sup> defghij	4.01 ± 0.35 <sup>ac</sup> defghij	2.88 ± 0.32 <sup>abc</sup> defghij
Total content	46.68 ± 3.61 <sup>bc</sup> defghij	39.92 ± 1.66 <sup>abc</sup> defghij	31.10 ± 1.18 <sup>abc</sup> defhij	19.16 ± 0.65 <sup>abc</sup> defghij	39.56 ± 1.95 <sup>abc</sup> defghij	30.44 ± 3.17 <sup>abc</sup> defghij	32.65 ± 2.99 <sup>abc</sup> defghij	23.17 ± 3.22 <sup>abc</sup> defghij	34.56 ± 2.21 <sup>abc</sup> defghij	24.38 ± 2.33 <sup>abc</sup> defghij

<sup>a</sup>p < 0.05 versus CNB cultures 30 days growth period

<sup>b</sup>p < 0.05 versus CNB cultures 60 days growth period

<sup>c</sup>p < 0.05 versus CTB cultures 30 days growth period

<sup>d</sup>p < 0.05 versus CTB cultures 60 days growth period

<sup>e</sup>p < 0.05 versus NSB cultures 30 days growth period

<sup>f</sup>p < 0.05 versus NSB cultures 60 days growth period

<sup>g</sup>p < 0.05 versus RITA® bioreactor cultures 30 days growth period

<sup>h</sup>p < 0.05 versus RITA® bioreactor cultures 60 days growth period

<sup>i</sup>p < 0.05 versus Plantform bioreactor cultures 30 days growth period

<sup>j</sup>p < 0.05 versus Plantform bioreactor cultures 60 days growth period

**Table 3** Flavonoid contents (mg 100 g<sup>-1</sup> DW ± SD) in extracts from biomasses of *Schisandra chinensis* microshoots cultivated in in different types of bioreactors (n = 4, p < 0.05)

Flavonoids	Bioreactor type																							
	CNB				CTB				NSB				RITA®				Plantform							
	Growth period (days)																							
	30			60			30			60			30			60			30			60		
Kaempferol	1.14 ± 0.07 <sup>def</sup>	1.05 ± 0.06 <sup>cdef</sup>	2.07 ± 0.06 <sup>abdehij</sup>	0.18 ± 0.02 <sup>abcdehij</sup>	6.49 ± 0.62 <sup>abcdehij</sup>	3.16 ± 0.08 <sup>abcdehij</sup>	1.11 ± 0.17 <sup>cdef</sup>	1.08 ± 0.28 <sup>cdef</sup>	0.98 ± 0.12 <sup>cdef</sup>	1.11 ± 0.17 <sup>cdef</sup>	1.23 ± 0.20 <sup>cdef</sup>	1.23 ± 0.08 <sup>abcdehij</sup>	1.23 ± 0.20 <sup>cdef</sup>	1.11 ± 0.17 <sup>cdef</sup>	1.08 ± 0.28 <sup>cdef</sup>	0.98 ± 0.12 <sup>cdef</sup>	1.11 ± 0.17 <sup>cdef</sup>	1.23 ± 0.20 <sup>cdef</sup>	1.23 ± 0.08 <sup>abcdehij</sup>	3.16 ± 0.08 <sup>abcdehij</sup>	6.49 ± 0.62 <sup>abcdehij</sup>	0.18 ± 0.02 <sup>abcdehij</sup>		
Quercitrin	18.39 ± 0.43 <sup>hij</sup>	17.71 ± 0.36 <sup>dehgh</sup>	18.34 ± 0.34 <sup>ghij</sup>	11.85 ± 0.09 <sup>abcdegi</sup>	21.00 ± 0.69 <sup>klmij</sup>	13.27 ± 0.22 <sup>abcdegi</sup>	12.55 ± 0.96 <sup>abcdegi</sup>	17.53 ± 0.85 <sup>dehij</sup>	13.25 ± 0.54 <sup>abcdegi</sup>	12.55 ± 0.96 <sup>abcdegi</sup>	18.52 ± 0.63 <sup>ghij</sup>	13.27 ± 0.22 <sup>abcdegi</sup>	18.52 ± 0.63 <sup>ghij</sup>	12.55 ± 0.96 <sup>abcdegi</sup>	17.53 ± 0.85 <sup>dehij</sup>	13.25 ± 0.54 <sup>abcdegi</sup>	12.55 ± 0.96 <sup>abcdegi</sup>	18.52 ± 0.63 <sup>ghij</sup>	13.27 ± 0.22 <sup>abcdegi</sup>	21.00 ± 0.69 <sup>klmij</sup>	11.85 ± 0.09 <sup>abcdegi</sup>			
Rutoside	1.26 ± 0.04 <sup>cdegi</sup>	1.25 ± 0.06 <sup>cdegi</sup>	1.62 ± 0.02 <sup>abdfj</sup>	0.88 ± 0.01 <sup>abcdehij</sup>	1.53 ± 0.07 <sup>abdfj</sup>	1.01 ± 0.03 <sup>bcdegi</sup>	1.27 ± 0.30 <sup>cdegi</sup>	1.87 ± 0.21 <sup>abdfhij</sup>	1.22 ± 0.73 <sup>cdegi</sup>	1.27 ± 0.30 <sup>cdegi</sup>	1.52 ± 0.21 <sup>abdfj</sup>	1.01 ± 0.03 <sup>bcdegi</sup>	1.52 ± 0.21 <sup>abdfj</sup>	1.27 ± 0.30 <sup>cdegi</sup>	1.87 ± 0.21 <sup>abdfhij</sup>	1.22 ± 0.73 <sup>cdegi</sup>	1.27 ± 0.30 <sup>cdegi</sup>	1.52 ± 0.21 <sup>abdfj</sup>	1.01 ± 0.03 <sup>bcdegi</sup>	1.53 ± 0.07 <sup>abdfj</sup>	0.88 ± 0.01 <sup>abcdehij</sup>			
Total content	20.78 ± 0.54 <sup>cdehij</sup>	20.00 ± 0.48 <sup>cdehij</sup>	22.03 ± 0.42 <sup>abdehij</sup>	12.90 ± 0.12 <sup>abcdehij</sup>	29.02 ± 1.38 <sup>abcdehij</sup>	17.44 ± 0.33 <sup>abcdehij</sup>	13.93 ± 1.43 <sup>abcdehij</sup>	20.48 ± 1.34 <sup>cdehij</sup>	15.45 ± 1.39 <sup>abcdehij</sup>	13.93 ± 1.43 <sup>abcdehij</sup>	21.27 ± 1.04 <sup>abdehij</sup>	17.44 ± 0.33 <sup>abcdehij</sup>	21.27 ± 1.04 <sup>abdehij</sup>	13.93 ± 1.43 <sup>abcdehij</sup>	20.48 ± 1.34 <sup>cdehij</sup>	15.45 ± 1.39 <sup>abcdehij</sup>	13.93 ± 1.43 <sup>abcdehij</sup>	21.27 ± 1.04 <sup>abdehij</sup>	17.44 ± 0.33 <sup>abcdehij</sup>	29.02 ± 1.38 <sup>abcdehij</sup>	12.90 ± 0.12 <sup>abcdehij</sup>			

<sup>a</sup>p < 0.05 versus CNB cultures 30 days growth period<sup>b</sup>p < 0.05 versus CNB cultures 60 days growth period<sup>c</sup>p < 0.05 versus CTB cultures 30 days growth period<sup>d</sup>p < 0.05 versus CTB cultures 60 days growth period<sup>e</sup>p < 0.05 versus NSB cultures 30 days growth period<sup>f</sup>p < 0.05 versus NSB cultures 60 days growth period<sup>g</sup>p < 0.05 versus RITA® bioreactor cultures 30 days growth period<sup>h</sup>p < 0.05 versus RITA® bioreactor cultures 60 days growth period<sup>i</sup>p < 0.05 versus Plantform bioreactor cultures 30 days growth period<sup>j</sup>p < 0.05 versus Plantform bioreactor cultures 60 days growth period

and 7.90, after 30- and 60-day growth cycles, respectively) (Table 1), but also in higher production of phenolic acids (Table 2). Phenolic compounds are the plant's "stress metabolites", hence the high production (Halliwell 2003, 2007; Akula and Ravishankar 2011). Moreover, it is worth noting that the 'bubble (air-lift) bioreactors' had been successfully applied before for the production of caffeic acid derivatives in *Echinacea purpurea* adventitious root cultures (Jeong et al. 2009), ginsenosides in adventitious root cultures of *Panax ginseng* (Kim et al. 2004) as well as phenolic acids and flavonoids in *Eleutherococcus senticosus* somatic embryos (Shohael et al. 2006).

In this study, the gas-phase nutrient sprinkle bioreactor (NSB) proved to be the best for flavonoid production (Table 3). The characteristic feature of this bioreactor design is the lack of mechanical stress exerted on growing biomass, as well as good gas circulation and nutrient accessibility (Steingroewer et al. 2013; Georgiev et al. 2014). Similar to our study, the correlative experiment on *Artemisia annua* in vitro shoots had showed an improvement in artemisinin output in a gas-phase bioreactor (Liu et al. 2006). Moreover, shoots grown in a nutrient sprinkle bioreactor have been employed for effective biomass growth and production of phenolic compounds, expressed as total phenolic acids and flavonoids, in medicinal plants such as *Rehmannia glutinosa* (Piąteczak et al. 2014) and *Scutellaria alpina* (Grzegorzczak-Karolak et al. 2017).

To conclude, our work showed that *S. chinensis* in vitro microshoots grown in different types of bioreactors (Fig. 1, Table 1), could provide biologically active phenolic compounds. Moreover, we proved, for the first time, the influence of bioreactor type on the phenolic acid and flavonoid production in *S. chinensis* biomass (Tables 2 and 3). The obtained results are thus a good starting point for further studies, involving scale-up experiments.

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**Author contributions** AS, AK, MB and KJ performed the research and elaborated the data. AK performed the bioreactor experiments. AS and MB performed the chemical analysis. AS performed the statistical analysis and prepared the manuscript. AK, ML and HE checked and corrected the manuscript.

## Compliance with ethical standards

**Conflict of interest** All authors approved the manuscript and declare that there are no conflicts of interests.

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