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Impact of photoselective colored nets on growth, anatomy, iridoid and phenolic content, and antioxidant capacity of the endangered medicinal plant *Stachytarpheta sellowiana* Schauer

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Abstract

Stachytarpheta sellowiana is a medicinal plant native to Brazil that is at risk of extinction. Light is one of the environmental factors directly influencing plant growth, anatomy, and metabolite production. The study aimed to assess the spectral influence of light transmitted through photoselective colored nets on the vegetative growth, anatomical features, metabolites (photosynthetic pigments, iridoid, phenols, and total flavonoids), and antioxidant capacity. The study followed a completely randomized experimental design, with four treatments: black, red, blue, and sunlight. Parameters leaf area, photosynthetic pigments, and length of the longest branch showed higher values in plants cultivated under blue and black nets. Plants grown under sunlight recorded higher dry weight accumulation. The best values recorded for parameters leaf area, photosynthetic pigments, and plant length were observed in plants grown under blue and black nets. In addition, significant differences were observed in anatomical features among all treatments. Plants grown under blue net accumulated larger amounts of total iridoids and showed higher antioxidant capacity. Photoselective colored nets changed the growth, anatomy, metabolite production, and antioxidant capacity. This species can be cultivated under sunlight to achieve higher dry weight accumulation. However, cultivation under blue nets is recommended for higher production of antioxidant compounds and iridoids.

Keywords Medicinal plant, Agronomic parameters, Anatomical features, Trichomes

1 Introduction

Light is an essential energy source for the photosynthesis process. Plant responses to the light energy-capturing process can change depending on the wavelength, intensity, and direction of the emissions. Moreover, light quality and intensity are crucial for energy



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conversion to take place, so that it can be available and captured through the photosynthesis process [1–3].

Light absorption results from the presence of phytochromes that trigger many specific physiological responses in plants [4, 5]. The spectral quality of incident and absorbed light can be improved by using photoselective colored nets, which represent an important agrotechnological strategy for manipulating plant growth and metabolism under protected cultivation, and it contributes to higher photosynthetic efficiency [6]. This alteration in light spectrum happens through wavelength manipulation by converting direct light into diffuse light, which can be beneficial to increase protected crop yield [7]. Furthermore, these changes can affect physiological and anatomical responses, growth, and development [8].

A new agrotechnological concept has been introduced to promote these changes in radiation emission and absorption, namely, using colored nets. These nets allow changing plants' physiological responses [1, 9–11], enabling the modulation of both primary and secondary metabolism without genetic manipulation. It is possible mentioning the black net case as an example of colored nets' effect, since it reduces the amount of light, whereas red and blue nets change light composition [12–15].

Plant growth can be altered by either its stimulus or inhibition, and leaf anatomy can be modified [9–11, 16–18]. The nets can also affect secondary compounds in plants, which is particularly relevant for medicinal species of ecological or pharmacological interest. According to Marafeli et al. [9], blue light spectra influence total cardenolides and glucoevatromonoside (GEV) in *Digitalis mariana*. *Lippia gracilis* plants cultivated under red and black ChromatiNet favored thymol, carvacrol, and (E)-caryophyllene contents [11].

Studies have shown the medicinal potential of species belonging to genus *Stachytarpheta*, due to their chemical composition [19–21]. Traditionally and experimentally, species of this genus have been reported as sources of bioactive compounds with anti-inflammatory, analgesic, antimicrobial, antimalarial, antihypertensive, and antidiabetic properties, among others [19–21].

Stachytarpheta sellowiana (Verbenaceae) is a plant native to Brazil and endemic to the Cerrado region [22, 23]. Given its conservation status and the increasing interest in the medicinal potential of the genus, developing sustainable strategies for biomass and metabolite production is essential to reduce pressure on natural populations.

No agronomic studies with *Stachytarpheta sellowiana* were found through the literature search. Additionally, there is a lack of information regarding the effects of colored nets on the growth and iridoid accumulation in this species. In this context, the establishment of cultivation strategies that promote plant development and secondary metabolite production under controlled conditions represents a relevant approach for both conservation purposes and future pharmacological investigations.

The present study is the first to report this plant's growth and iridoid production response to different wavelengths. The study hypothesis advocates that wavelength variations could influence both biomass production and secondary metabolism in plants under colored nets. Thus, the current research aimed to assess the effects of light spectrum transmitted through colored nets on the vegetative growth, anatomical features, metabolite production (photosynthetic pigments, iridoids, phenols, and total flavonoids), and antioxidant capacity.

2 Materials and methods

2.1 Vegetable growth

Stachytarpheta sellowiana was registered on the SisGen Platform (Registration A97517C), in compliance with the Brazilian Biodiversity Law No. 13,123/2015. The voucher specimen of the parent plant was deposited in the herbarium of the Agricultural Research Company of Minas Gerais (EPAMIG), under registration number. PAMG 58,889.

The mother plants were cultivated in the Medicinal Garden of Federal University of Lavras (UFLA), geographic coordinates 21° 14' 43" S and 44° 59' 59" W, and elevation of 918 m. Seedlings were produced from nodal cuttings that were taken from the second to the fourth node. After 90 days, seedlings were transplanted into 10-liter plastic pots—one plant per pot. The used soil consisted of subsoil horizon b (dystrophic red latosol) and sand at a 1:1 (v/v) ratio—12 kg of cattle manure per m² was added to the mix.

The pots were maintained in protected environments under black, blue, and red shade nets, as well as under full sunlight (control). The colored nets were ChromatiNet® (Poly-sack Indústrias, Leme, SP, Brazil) and provided 50% shading. The light spectra under the nets were recorded in a handheld spectrometer, SPECTRA PEN Z850 (Qubit Systems-Kingston, Ontario, Canada). The radiation intensity was measured using a LI-185 quantummeter-photometer-radiometer (LI-COR Biosciences, Lincoln, NE, USA) equipped with a quantum sensor. The study followed a completely randomized experimental design, with four treatments, five replicates, and five plants per replicate, for a total of 100 plants.

This plant exhibits slow growth, which explains the 300-day post-transplanting time established for harvesting. This timeframe was defined based on plant development observations, according to which plants had shown higher growth stability and uniformity. The following parameters were determined: shoot length (SL), leaf (LDW), stem (SDW), root (RDW), total dry weight (TDW), and total leaf area (TLA). Plants were harvested and partitioned into leaves, stems, and roots to determine the dry weight (g). Subsequently, they were dried in an air-circulating oven at 45 °C, until the material reached constant weight. Total leaf area (TLA—cm²) was measured using an EPSON PERFECTION V700 PHOTO scanner, and quantified with WinFOLIA software. Five plants representative of each treatment were selected, and the leaf area of 100 leaves was assessed. The following parameters were calculated: leaf area ratio (LAR = total leaf area: total plant dry weight), specific leaf area (SLA = total leaf area: plant leaf dry weight), and leaf weight ratio (LWR = leaf dry weight: total plant dry weight) [24, 25].

2.2 Analysis of photosynthetic pigments

The extraction and quantification of photosynthetic pigments followed the method developed by Hiscox and Israelstam [26], with adaptations proposed by Barnes et al. [27]. Fifty milligrams of fresh leaves were weighed directly into Falcon tubes wrapped in aluminum foil. Subsequently, 10 mL of dimethyl sulfoxide (DMSO) saturated with calcium carbonate (CaCO₃) was added, and the samples were incubated at 65 °C for 48 h. The saturation of DMSO with calcium carbonate (CaCO₃) followed the protocol described by Santos et al. [28]: DMSO was saturated with CaCO₃ at a concentration of 5 g L⁻¹ under constant stirring for 4 h, vacuum-filtered twice through double filter paper, and then centrifuged at 6000 rpm for 10 min. All samples were prepared in quintuplicate.

Wavelengths and equations used in the calculations followed the methodology by Wellburn (1994):

$$\text{Chlorophyll } a649 = (12 \cdot 47 \times A665) - (3 \cdot 62 \times A649);$$

$$\text{Chlorophyll } b665 = (25 \cdot 06 \times A649) - (6 \cdot 5 \times A665);$$

$$\text{Carotenoids } 480 : (1000 \times A480 - 1 \cdot 29 \times Ca - 53 \cdot 78 \times Cb)/220$$

Total chlorophyll (a + b) was calculated by summing the results of the equations for chlorophyll a and b. All results are expressed in mg g^{-1} fresh matter.

2.3 Phenolic compound levels and antioxidant capacity

2.3.1 Extract preparation and general conditions

Dehydrated leaves were ground in knife mill and sieved in 20-mesh sieve. The aliquot of 50 mg ground dry leaves was weighed in microtubes and 2 mL MeOH: H₂O (1:1) were added to it. This process was followed by homogenization in vortex for 5 s. These samples were subjected to extraction by sonication (three cycles, 15 min each). The extracts were microcentrifuged at 12,000 rpm for 10 min after each extraction cycle; the supernatants were collected, combined, and rotary evaporated at 40 °C until drying. The extracts were resuspended in 1 mL alcohol and water solvent (1:1 ratio) to proceed with the readings. Molecular absorption readings within the ultraviolet range were measured in a TECAN INFINITY M200 PRO microplate reader, operated with an I-Control® data processing system, version 3.37. All treatments were assessed in quintuplicate.

2.3.2 Total phenols

The quantification of total phenolic compounds was performed through the Folin-Ciocalteu method, following the methodology described by Singleton et al. [29], with modifications described by Coelho et al. (2021). The calibration curve was plotted by using gallic acid (Sigma-Aldrich®, ≥ 98%) standards ranging from 0.7188–1.2316 mg mL^{-1} to generate the equation: $y = 2.2178 \times - 0.1251$ ($R^2 = 0.9986$). Results were expressed in milligrams of gallic acid equivalents per gram of leaf dry weight (mg GAE g^{-1} DW).

2.3.3 Total flavonoids

The quantification of total flavones and flavonols in the extracts was performed according to the methodology by Atanassova et al. [30], with modifications described by Coelho et al. 2021. Results were expressed as milligrams of quercetin equivalents per gram of leaf dry weight (mg QE g^{-1} LDW).

2.3.4 Total antioxidant capacity (TAC)

Total antioxidant capacity (TAC) was assessed based on the molybdate reduction methodology [31], with the following modifications described by Coelho et al., 2021. A calibration curve was of ascorbic acid (Sigma-Aldrich® 99.7%) at concentrations ranging from 0.3587–0.6470 mg mL^{-1} —the line equation was $y = 16.544 \times - 0.1162$ ($R^2 = 0.9946$). Results were expressed in milligrams of ascorbic acid equivalents per gram of leaf dry weight (mg AAE g^{-1} DW).

2.4 Total iridoid quantitation

2.4.1 Plant material, chemicals, and equipment

The plant material comprised leaves dehydrated in an air-circulating oven at 38 ± 2 °C, and ground in a knife micro-mill (Marconi, Model MAO 48, Brazil) with a 35-mesh sieve. Methanol (Sciavicco®, 99.95%), copper sulfate pentahydrate (Êxodo®, 98.0%), hydrochloric acid (Vetec®, 37%), and aucubin (HWI Pharma®, Germany, 94.8%) were used for the colorimetric reaction. Ultraviolet molecular absorption readings were taken in TECAN Infinity® M200 PRO spectrophotometer operated by I-control® software, version 3.37.

2.4.2 Extraction optimization and sample preparation

Several iridoid extraction methods have been described in the literature [32–35]. An optimal condition was first determined to extract the maximum amount of iridoids from dried leaves because there was no consensus about it in the literature. Different extractor solvents (70% ethanol, 70% methanol, and 10% aqueous KOH solution), and their binary and ternary combinations, were assessed through simplex-centroid mixture design and surface response methodology, as described by dos Santos et al. [36]. See Table S1, in supplementary material, for extraction planning details.

The established extraction method comprised sonication for 10 min, using 10 mg powdered leaves in 1500 µL 70% methanol (Fig. S1). The extracts were centrifuged at 12,000 rpm for 10 min; the supernatant was collected, and the solvent was evaporated to dryness in a rotary evaporator at 40 °C. The residue was resuspended in exactly 1000 µL 70% methanol (sample solution).

2.4.3 Spectrophotometric quantification

Total iridoid concentration was assayed by measuring the absorbance of the product resulting from Trim-Hill reagent, at 622 nm, according to the method described by Trim and Hill [37]. This method was validated by following the guidelines by the International Conference on Harmonization [38] and Brazil [39]. It was done by assessing selectivity, precision, recovery, linearity, limit of quantification (LOQ), limit of detection (LOD), and robustness.

The sample solution was transferred to a 10 mL volumetric flask, and the volume was completed through Trim-Hill reagent addition. The reading solution was transferred to a test tube and placed in a water bath at 100 °C for 5 min; this procedure was followed by cooling in a water bath at 25 °C for 2 min. Six aliquots of 280 µL reading solution were transferred to six wells in a polystyrene microplate. The aliquot of 10 mL volumetric flasks, a blank solution, and compensation solutions were prepared separately. The blank solution was prepared by transferring 1000 µL 70% methanol and by completing the volume through Trim-Hill reagent addition. The compensation solution was prepared by transferring 1 mL sample solution and by adjusting the volume with 70% methanol addition. The compensation solution was read against 70% methanol. The absorbance value recorded for the compensation solution was subtracted from the sample-reading results. All readings were performed in triplicate, and tests were conducted in sextuplicate. A calibration curve was of aucubin (Sigma-Aldrich® 99.7%) at concentrations ranging from 0.0125–0.2 mg mL⁻¹—the line equation was $y = 7.5597 \times - 0.0146$ ($R^2 = 0.9968$).

Results were expressed in milligrams of aucubin equivalents per gram of leaf dry weight (mgAE gLDW⁻¹).

2.5 Scanning electron microscopy (SEM)

Samples were prepared by following the methodology described by Alves et al. [40], with modifications described by Ribeiro et al., 2024. The following parameters were measured: number of tector trichomes (NTT), number of septate glandular trichomes (NSGT) and number of peltate glandular trichomes (NPGT), number of stomata (NS) per unit area (mm²), and stomata polar (PDS) and equatorial (EDS) diameters.

2.6 Statistical analysis

After assessing the experiments, data were collected and subjected to analysis of variance - means were compared through Scott-Knott test, at 5% probability level, in SISVAR 5.6 software [41]. Software Statistica®, version 13.3 (StatSoft, Tulsa, OK, USA), was used in principal component analysis (PCA).

2.7 Results and discussion

2.7.1 Vegetable growth

The use of photoselective colored nets can affect plant biomass. These nets are commonly used in protected cultivation. They filter sunlight and change the light spectrum reaching the plants, which influences processes such as photosynthesis, transpiration, and overall plant development. Photoselective colored nets significantly influenced *Stachytarpheta sellowiana* biometric response variables in comparison to the control (sunlight) (Fig. 1). Plant length (PL) showed higher values when plants were grown under red net (100.60 cm) and blue net (100.30 cm); there were no significant differences between them.

Figure 2A shows that plants grown under blue, red, and black nets showed greater elongation than those grown under sunlight. Plants grown in low-light environments tend to invest more in height growth due to the high activity of auxins and the search for light. This process is called etiolation [42]. This finding showed that light quality can change plant morphology, such as height, number of leaves, and leaf density, which affect biomass production. In addition, changes in wavelength can influence growth hormones, such as auxins and gibberellins, and affect biomass accumulation.

It was observed that plants grown under sunlight showed the highest dry weight accumulation increases, regardless of plant organ (Fig. 1). Only stem dry weight (SDW) observed for plants grown under red net was not statistically different from that of plants grown under sunlight. Dry weight accumulation in plants grown under sunlight is likely related to the high-light-intensity environment the plant is adapted to. These plants' native habitat includes rocky fields and the *cerrado* biome, where they receive abundant light throughout the day. On the other hand, the lower dry weight values recorded for plants grown under nets can be explained by radiation reduction, since there may be decrease in signals regulating plant development in low-light environments. This process has impact on nutrient assimilation and translocation, besides affecting plants' morphological, anatomical and physiological features [43].

Figure 1D shows a larger root volume under the sunlight treatment. Root dry weight (RDW) under sunlight was 73%, 93% and 2.3 times higher than that recorded for plants

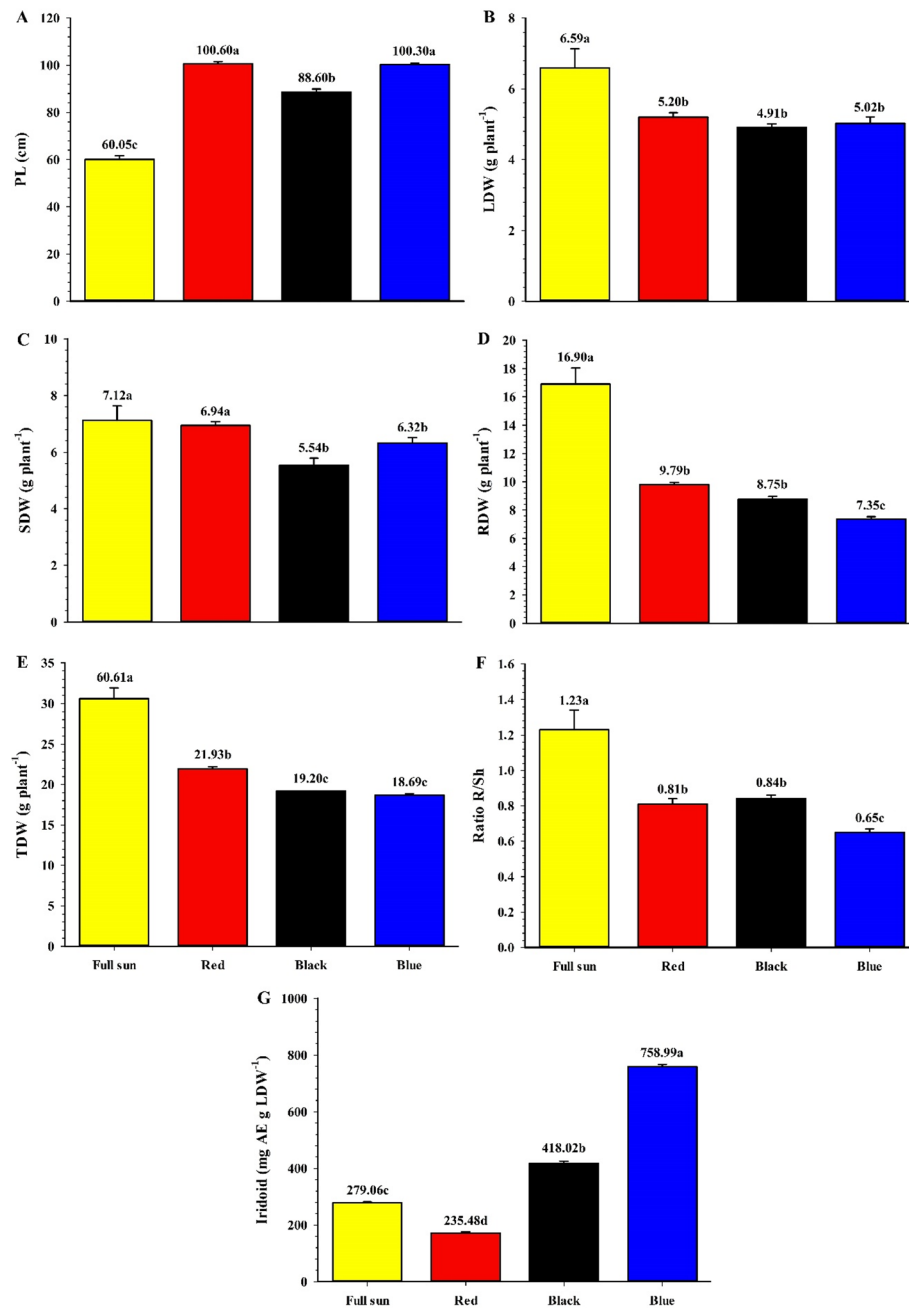


Fig. 1 **A** Plant length (PL), **B** leaf dry weight (LDW), **C** stem dry weight (SDW), **D** root dry weight (RDW), **E**: total dry weight (TDW), and **F** root-to-shoot ratio (R: Sh) **G** analysis of iridoids content of *Stachytarpheta sellowiana* grown under full sunlight and red, black and blue nets. The mean values followed by the same letter do not differ by the Scott–Knott test ($p < 0.05$). Vertical bars represent the standard deviation

grown under red, black, and blue nets, respectively. Figure 2B shows dry weight (%) distribution among different organs of plants grown under different net colors and under sunlight. Figure 2B presents the root: shoot ratio of plants grown under different net colors and sunlight. Most of the biomass of plants grown under sunlight was accumulated in the roots (55%), whereas the highest accumulation occurred in the shoots of plants grown under blue net (61%). However, biomass distribution between organs was more balanced in plants grown under the red and black nets.

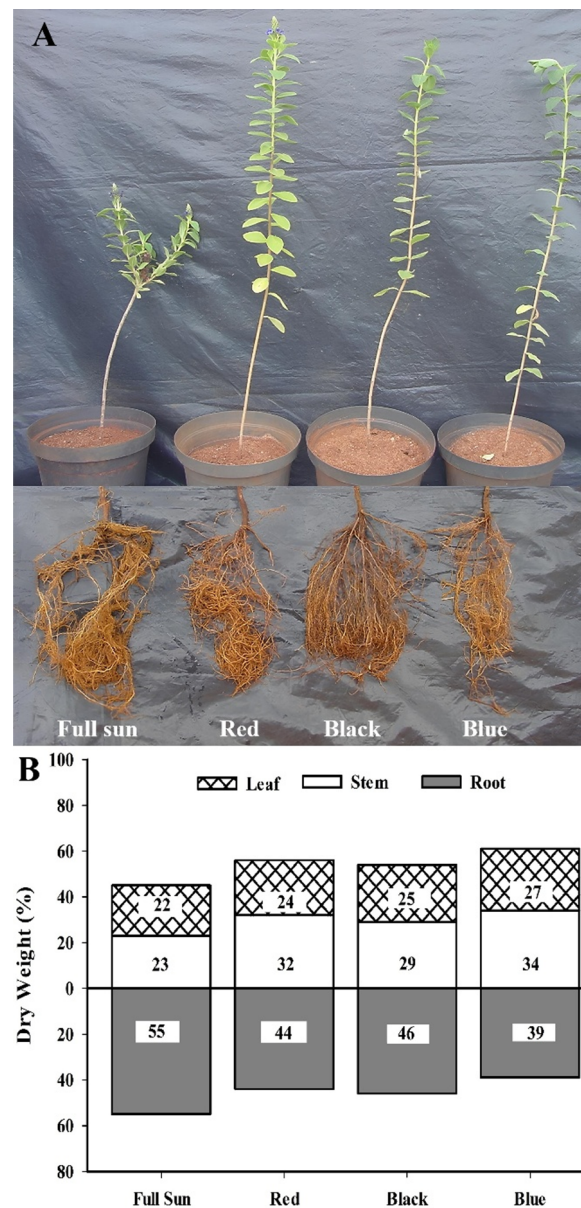


Fig. 2 **A** *Stachytarpheta sellowiana* plants grown and **B** distribution of dry weight (%) among the different organs, under different net colors and in sunlight

The species' growth under colored nets intensely depends on genotype; each species potentially responds differently to each cultivation environment. Alves et al. [44] detected higher root dry weight in *Lippia alba* grown under sunlight and higher leaf dry weight in plants grown under blue net cultivation. However, Ribeiro et al. [1] reported that the cultivation of *Pogostemon cablin* under red nets resulted in higher leaf, root, and stem dry weight and total biomass production. On the other hand, *Lippia gracilis* accumulated higher leaf and stem dry weight under red, black, and blue nets [11]. The best response to cultivation under colored nets recorded for *Digitalis mariana* was observed under the blue net [9].

Red and blue light spectra may have had an impact on plant growth, since they are the main photosynthetic CO₂ assimilation source [45, 46]. Furthermore, the species

presented etiolation due to low light intensity. The high energy efficiency of the Calvin cycle points out that some regulation forms ensure that all intermediates are at adequate concentrations and that the cycle turns off when it is not needed, during the dark period. Variations in concentration or enzymatic activity can modulate catalytic rates and change metabolite levels in the cycle.

The highest mean leaf area was found in plants grown under the blue net (13.06 cm²), whereas plants grown under sunlight showed 57.6% leaf area reduction in comparison to plants grown under the blue net (6.99 cm²) (Table 1). Leaves develop different features in shaded environments. There is an incentive to increase leaf area to optimize photosynthesis; lower light levels lead to better use of low light intensity [47].

Plants in shaded environments invest larger proportions of photoassimilates to increase leaf area and maximize light capture. They often have thin leaves with higher specific leaf area (SLA) [48, 49]. This phenomenon supports the results in the present study, because the treatments promoting a larger leaf area also achieved higher specific leaf area (blue 2.60 cm² g⁻¹ and red net 2.16 cm² g⁻¹, Table 1). Higher specific leaf area implies thinner leaves. Specific leaf area (SLA) can be used to estimate photosynthetic assimilation rates. Lower SLA value (1.16 cm² g⁻¹) is observed in plants subjected to sunlight cultivation in comparison to those grown under nets. This finding suggests higher leaf dry weight retention in sunlight environments than under nets. This outcome was evidenced by higher leaf dry weight accumulation under sunlight (Fig. 1B).

Specific leaf area (SLA) determination was calculated as leaf area: leaf dry weight ratio (SLA = TLA: LDW). This parameter can be applied to quantify acclimation strategies, mainly the differential allocation of resources for photosynthesis in response to environmental conditions [48, 50–52]. Specific leaf area indicates whether plants adopt conservative or acquisitive strategies to use environmental resources [48, 53]. Moreover, environmental conditions determine the redistribution of nutritional resources to tissues, and it enables plant growth and defense under environmental stress [54–57].

Benincasa [24] reported that SLA inverse indicates leaf thickness, which is the specific leaf weight (SLW = LDW: TLA). Higher SLW (0.91 g cm⁻²) was observed in plants grown under sunlight, whereas the lowest SLW (0.41 g cm⁻²) was observed under the blue net; therefore, plants presented higher and lower leaf thickness, respectively. Ribeiro et al. [1] found higher leaf thickness in *Pogostemon cablin* when it was cultivated under red nets. Leaves developing under low light intensity tend to be thinner and to have lower leaf dry weight; this feature increases their specific leaf area and, consequently, light interception [58]. These data support the lower dry weight accumulation observed in *Stachytarpheta sellowiana* plants, as shown in Fig. 1. The high SLA value recorded in the low-light environment can be related to reduced investment in structures, such as the epidermis, which helps plants' protective mechanisms against photoinhibition [59].

Table 1 Leaf traits analysis of *Stachytarpheta sellowiana* plants grown under different types of colored nets and full sun

Treatments	TLA (cm ²)	SLA (cm ² /g)	SLW g/cm ²	LAR (cm ² /g)	LWR (g/g)
Sunlight	7.5 ± 0.17 ^b	1.2 ± 0.07 ^b	0.9 ± 0.07 ^a	0.2 ± 0.01 ^d	0.5 ± 0.03 ^a
Red	10.3 ± 0.89 ^b	2.2 ± 0.37 ^a	0.5 ± 0.07 ^c	0.5 ± 0.05 ^b	0.4 ± 0.03 ^a
Black	7.0 ± 1.02 ^b	1.5 ± 0.39 ^b	0.7 ± 0.13 ^b	0.4 ± 0.01 ^c	0.5 ± 0.01 ^a
Blue	13.1 ± 1.16 ^a	2.6 ± 0.32 ^a	0.4 ± 0.03 ^c	0.7 ± 0.02 ^a	0.4 ± 0.02 ^a

TLA: Total leaf area; SLA: Specific leaf area; SLW: Specific leaf weight; LAR: Leaf area ratio; LWR: Leaf weight ratio. Averages followed by the same letters do not differ from each other at the 5% probability level using the Skot Knott test

Increase in leaf area ratio (LAR) in plants grown under the blue net ($0.69 \text{ cm}^2 \cdot \text{g}^{-1}$) was three times higher than that observed in plants grown under sunlight (Table 1). This factor can be a strategy adopted by plants to adapt to lower wavelengths. This finding indicates that *Stachytarpheta sellowiana* plants grown under nets require larger leaf area to produce a certain amount of dry weight in comparison to plants grown under sunlight. Chagas et al. [60] found that *Mentha piperita* L. plants grown under red and blue nets had larger leaf areas in the second harvest. Larger-area leaves often have thinner structure, and it increases their surface area for sunlight contact; it compensates the low light intensity [61].

Leaves produce 90% assimilates and translocate them throughout the plant. Benincasa [24] reported that the leaf weight ratio ($\text{LWR} = \text{LDW} : \text{LDW} + \text{SDW}$) expresses the dry weight fraction that is not exported from the leaves to the rest of the plant. This higher or lower leaf weight export is a genetic trait influenced by environmental variations. Table 1 showed no significant difference among cultivation environments. Thus, leaf tolerance to shading can result in organs adapted to different environmental conditions (sun and shade leaves) [62, 63].

2.7.2 Iridoid content

This study is the first to report the quantification of this class of compounds in *S. sellowiana* by taking into consideration the phytochemistry of the genus *Stachytarpheta*. Iridoids are monoterpenoid compounds distributed in several plant groups as secondary metabolism compounds [64].

Iridoid glycosides have been considered active constituents in several medicinal plants related to anti-inflammatory activity, such as *Harpagophytum procumbens* (Burch.) DC. ex Meisn, *Stachytarpheta cayennensis* (L.C. Rich) Vahl and *Scrophularia scorodonia* L [65]. Ipolamiide and some derivatives are currently acknowledged as valuable resources for the pharmaceutical sector, since they work as potential key starting materials for chemical synthesis and biosynthesis.

Total iridoid quantification in *Stachytarpheta sellowiana* was achieved by using a validated colorimetric method based on the Trim and Hill reaction. Iridoid glycosides, such as aucubin and some derivatives, are acid-labile and yield blue-colored acid-degradation products in the presence of a small amount of Cu^{++} , in glacial acetic acid [37]. The selectivity of this method was assessed by wavelength scanning (from 230 to 730 nm) of leaf extract and leaf extract + Trim-Hill reagent. The 622-nm wavelength was selected because it recorded the highest molecular absorption intensity, without any interference (Fig. S2). Method robustness was analyzed by employing both the established conditions and the intentional variations in wavelength reading (620 and 624 nm), incubation time (4 and 6 min), incubation temperature (90°C) and Trim-Hill reagent volume (8.75 and 9.25 mL). The method is robust to achieve small changes in wavelength reading; however, the established conditions must be strictly followed when it comes to the other assessed parameters. The linear regression analysis showed an excellent linear fit, with an R-squared value above 0.99 in the range 0.0125–0.2 mg/mL. This finding highlighted that iridoid levels can be accurately determined through this method. Relative standard deviation values below 5% evidenced that the method shows both intraday and interday accuracy [39]. Table S2 presents the overall results of the analytical validation in order to ensure method reliability.

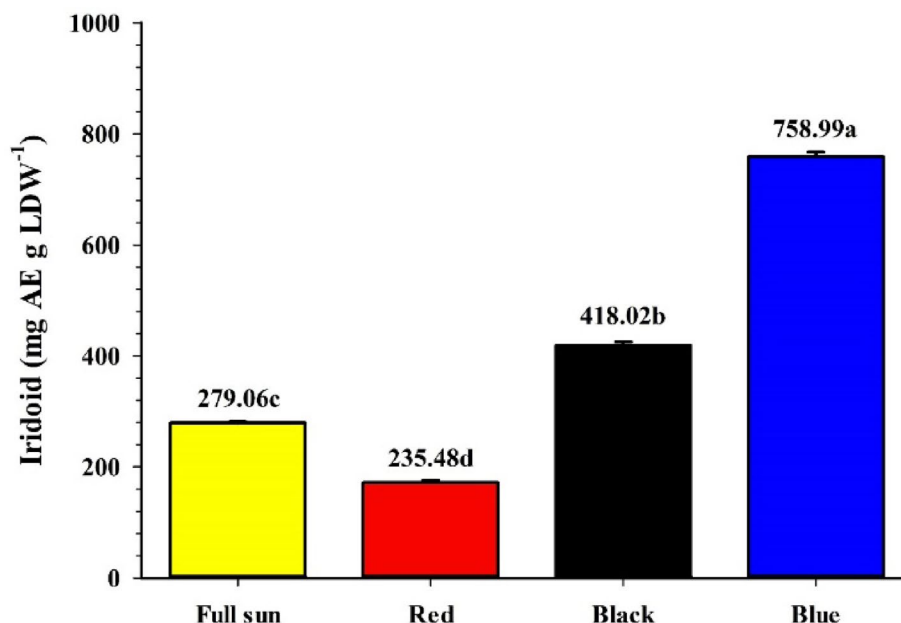


Fig. 3 Analysis of iridoids content of *Stachytarpheta sellowiana* grown under full sunlight and red, black and blue nets. The mean values followed by the same letter do not differ by the Scott–Knott test ($p < 0.05$). Vertical bars represent the standard deviation

Table 2 Quantification of photosynthetic pigments, analysis of total phenols, flavonoids and antioxidant tests of *Stachytarpheta sellowiana* plants, grown under colored nets and full sun

Treatments	Chlorophyll				TF	TFla	TAC
	Chl a (mg/g)	Chl b	Chl T	Carot			
Sunlight	0.57±0.05 ^b	0.14±0.02 ^c	0.72±0.07 ^c	0.17±0.01 ^b	66.5±3.2 ^a	0.70±0.01 ^a	90.1±3.1 ^b
Red	0.61±0.02 ^b	0.17±0.02 ^b	0.78±0.04 ^b	0.16±0.01 ^c	53.4±1.3 ^b	0.48±0.02 ^b	81.6±1.7 ^c
Black	0.74±0.06 ^a	0.20±0.02 ^a	0.93±0.07 ^a	0.19±0.01 ^a	36.6±1.2 ^c	0.48±0.01 ^b	75.8±3.6 ^d
Blue	0.75±0.03 ^a	0.19±0.02 ^a	0.94±0.05 ^a	0.18±0.01 ^a	51.8±2.2 ^b	0.41±0.01 ^c	105.6±2.5 ^a

Chl a: Chlorophyll a; Chl b: Chlorophyll b; Chl T: Chlorophyll total; Carot: Carotenoids; TF: Total phenols expressed in milligrams of gallic acid equivalent per gram of leaf dry weight (mgGAE g LDW⁻¹); TFla: total flavonoids expressed as milligram equivalent of quercetin per gram of leaf dry weight (mgQE g LDW⁻¹); TAC: total antioxidant capacity expressed in milligrams of ascorbic acid equivalents per gram of leaf dry weight (mgAAE g LDW⁻¹). Averages followed by the same letters do not differ from each other at the 5% probability level using the Skot Knott test

The total iridoid content significantly varied depending on the photoconverter net used in *Stachytarpheta sellowiana* cultivation (Fig. 3). The blue net provided higher iridoid content (values 3.22 and 2.72 times higher, respectively) than those grown under the red net and sunlight, which resulted in the lowest iridoid accumulation. Higashiuchi et al. [66] showed the effects of light intensity and light/dark period on iridoid concentration in *Hedyotis diffusa* plants grown in a chamber. The highest asperuloside content (an iridoid) was observed at 24/0 h under high light intensity.

2.7.3 Photosynthetic pigments, phenolic compound contents and antioxidant capacity

Plant growth efficiency is related to its ability to adapt to environmental light conditions. Quantitative photosynthetic pigment determination per unit of fresh weight showed that cultivation under blue and black nets led to increased chlorophyll a, b, total chlorophyll, and carotenoid concentrations in *Stachytarpheta sellowiana* leaves (Table 2). These findings are consistent with those observed by Oliveira et al. [13], according to whom, *Melissa officinalis* showed higher chlorophyll values when it was cultivated under

blue nets. Viana et al. [11] reported that *Lippia gracilis* plants recorded higher chlorophyll *a* and *b*, total chlorophyll, and carotenoid levels under the blue net and Marafeli et al. [9] found that *Digitalis mariana* plants also recorded higher chlorophyll *a* and *b*, total chlorophyll, and carotenoid levels under the blue net. According to Almeida et al. [67], higher chlorophyll accumulation under shading can compensate for the lower amount of available radiation. Thus, light quality affects photosynthesis, and it requires plants to adapt to a given environment under a specific light wavelength. This process is known as photosynthetic acclimation to light [68].

Carotenoids are found in all photosynthetic species. They absorb light at the 400–500 nm range, which gives them their orange color. They are considered accessory pigments because the light they absorb is transferred to the chlorophylls to drive the photosynthesis process. Yet, they are referred to as photoprotective agents against oxidative damage.

Plants are highly exposed to damage caused by adverse environmental conditions that have negative effects on their productivity, development, and survival. These harmful conditions increase the content of reactive oxygen species (ROS), such as O₂–(super-oxide) radicals, OH (hydroxyl) radicals, O₂ (singlet oxygen), and H₂O₂ (hydrogen peroxide) [69, 70]. Considering the physiological relevance of phenols and flavonoids in plant antioxidant defense, as well as their potential protective and therapeutic properties, phenolic compounds, flavonoids, total iridoids, and antioxidant capacity were evaluated in *Stachytarpheta sellowiana* plants grown under different colored shade nets.

The highest phenol (66.5 mg g^{−1}) and flavonoid (0.70 mg g^{−1}) levels were observed in the sunlight treatment (Table 2). Light plays key role in the biosynthesis of many antioxidants, such as carotenoids, flavonoids, anthocyanins, among others [71]. Leaves can have high flavonoid levels (flavones and flavonols) that help protecting their cells against excessive radiation. Plants' increased exposure to UV-B light results in higher flavone and flavonol synthesis [72, 73]. Studies have shown that plants grown under direct sunlight have higher phenolic substances and flavonoid levels, and it highlights the importance of UV radiation for flavonoids' biosynthesis. In addition, there is a well-established positive correlation between light intensity and phenolic substance production.

Table 2 showed that the highest TAC levels were observed under the blue net. The TAC method is based on reducing molybdenum (VI) to molybdenum (V) through antioxidants, and it is followed by the formation of a green phosphate complex of Mo (V) in acidic pH. Electron transfer in this assay depends on the antioxidant structure [31].

2.7.4 Scanning electron microscopy

Stachytarpheta sellowiana cultivation under different light conditions resulted in significant anatomical changes in the leaf blade (Fig. 4; Table 3). Glandular capitate and non-glandular trichomes, as well as stomata, were observed on both epidermal surfaces, which classified the leaf as amphistomatic. These structures are more abundant on the abaxial surface (Table 3).

S. sellowiana leaves presented capitate and peltate trichomes, mainly diacytic stomata (Fig. 4). The number of tector trichomes (NTT) on the adaxial surface was larger under the black net (269.05). In contrast, it was lower (284.53) on the abaxial surface in comparison to the other treatments (Table 3). The number of septate glandular trichomes (NSGT) on both the adaxial and abaxial surfaces was larger in treatments with red and

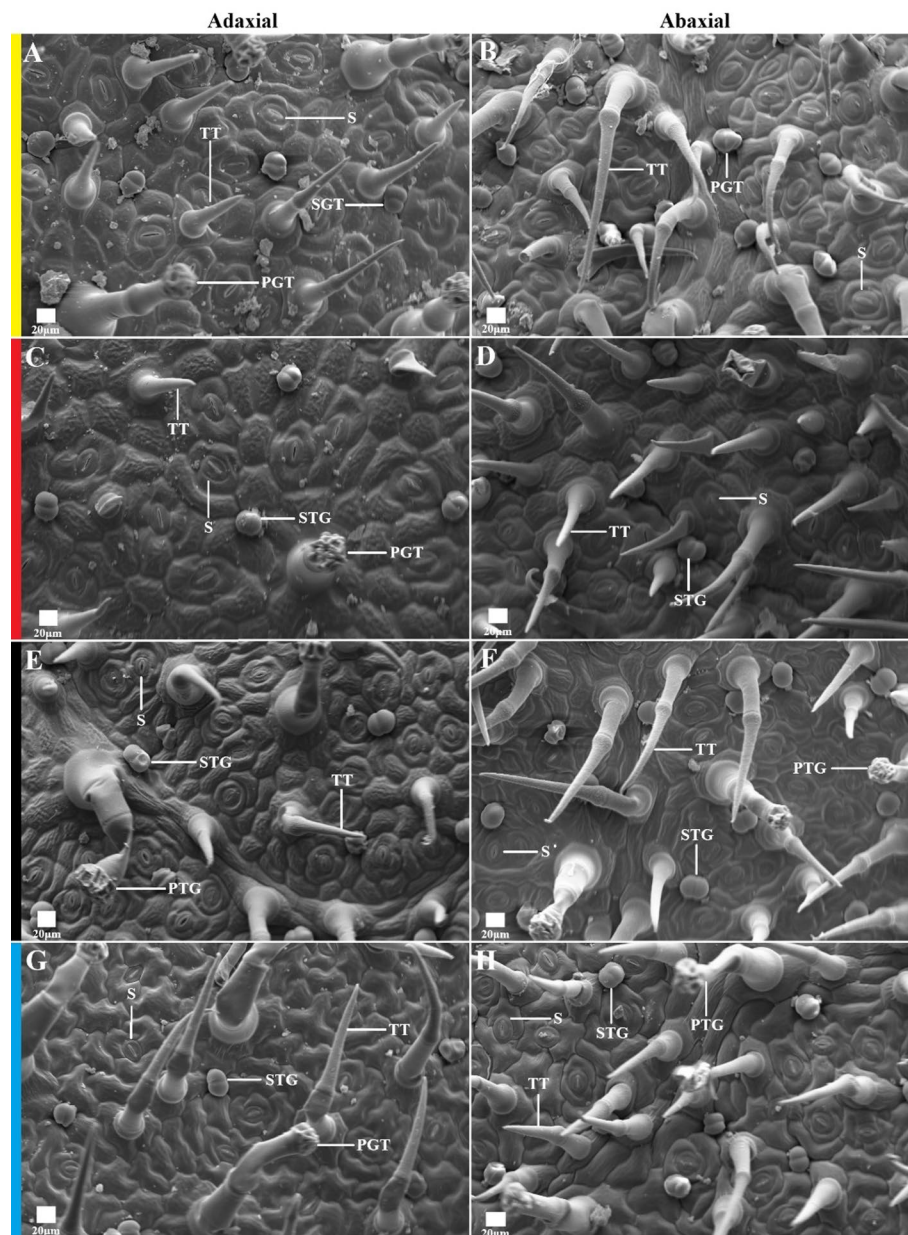


Fig. 4 *Stachytarpheta sellowiana*: Scanning electron micrograph of the adaxial (A, C, E, G) and abaxial (B, D, F, H) surface of the leaves

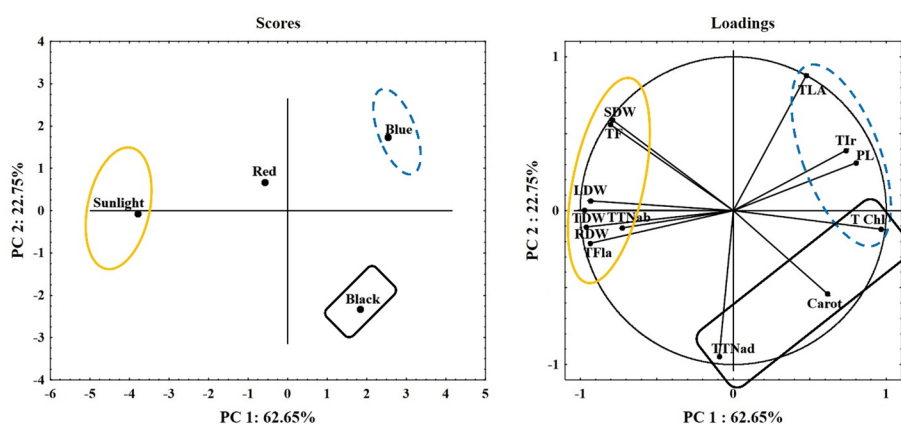
black nets (Table 3). The number of peltate glandular trichomes (NPGT) showed no statistical difference on either the adaxial or abaxial surfaces. The number of stomata (NS) on both the adaxial and abaxial surfaces was larger under the black mesh (632.75 and 896.04, respectively) (Table 3).

The equatorial diameter (EDS) and polar diameter (PDS) of stomata on the adaxial surface were larger under sunlight (177.81 μm and 191.79 μm , respectively) (Table 3). Stomata on the abaxial surface recorded larger equatorial and polar diameters under the black net (144.80 μm and 172.94 μm , respectively) (Table 3). According to Oren-Shamir et al. [74], the black net can be neutral because it only helps reduce radiation incidence on plants, without affecting radiation quality. Photomorphogenic responses, which

Table 3 Trichomes and stomata characteristics of *Stachytarpheta sellowiana* plants, grown under colored nets and full sun

Treatment	NTT	NSGT	NPGT	NS	EDS (μm)	PDS (μm)	PDS/EDS
Adaxial							
Full sun	169.9 b	114.7 b	42.5 a	348.2 b	177.8 a	191.8 a	1.2 a
Red	140.1 b	152.9 a	46.7 a	411.9 b	107.5 b	129.9 b	1.1 a
Black	269.0 a	144.4 a	29.7 a	632.7 a	113.6 b	132.5 b	1.2 a
Blue	97.7 b	101.9 b	17.0 a	314.2 b	124.6 b	143.2 b	1.1 a
Abaxial							
Full sun	445.9 a	152.9 b	59.4 a	595.5 c	89.8 b	104.7 b	1.2 a
Red	411.9 a	216.6 a	76.4 a	798.4 b	95.2 b	115.3 b	1.1 a
Black	284.5 b	242.1 a	63.7 a	896.0 a	144.8 a	172.9 a	1.2 a
Blue	407.7 a	80.7 c	34.0 a	450.1 d	93.1 b	106.0 b	1.2 a

NTT: Number of tector trichomes, NSGT: Number of septate glandular trichomes, NPGT: Number of peltate glandular trichomes; NS: Number of stomata; EDS: Equatorial diameter of the stoma; PDS: Polar diameter of the stoma. Averages followed by the same letters do not differ from each other at the 5% probability level using the Skot Knott test

**Fig. 5** Scores and loadings graph of principal component analysis (PCA), in the construction of matrix correlation, using data on Leaf Dry Weight (LDW); Root Dry Weight (RDW); Stem Dry Weight (SDW) Total Dry Weight (TDW); Flavonoids, Phenols, Iridoid Content, Carotenoids, Total Chlorophyll; Total Trichome Number abaxial and adaxial (TTNab and TTNad) and Total Leaf Area (TLA) of *Stachytarpheta sellowiana* grown under colored nets and full sun

include stomatal development, result from a complex network of light-sensitive physiological processes and are directly influenced by it [75].

The stomatal polar diameter: equatorial diameter ratio (PDS: EDS) showed no statistical differences; however, higher values were observed for this parameter under sunlight and black net, both on the adaxial and abaxial surfaces (1.18, 1.18, 1.22, and 1.22, respectively). The higher the PDS: EDS ratio, the more elliptical the stomata, and the more elliptical they are, the greater their functionality [76].

2.7.5 Principal component analysis (PCA)

PCA was applied to 13 data points to identify interactions between response variables and the colored nets. Figure 5 shows the correlation matrix among vegetative growth (total leaf area—TLA, plant length—PL, leaf—LDW, stem—SDW, root—RDW and total—TDW dry weight), flavonoids, phenols, photosynthetic pigments (total chlorophyll, carotenoids), iridoids and total trichomes number abaxial and adaxial (TTNab and TTNad), to explain 85.40% total variation. Thus, PC1 and PC2 allowed identifying treatments' separation into three groups to observe differences between plants: Group

1—plants grown under sunligh; Group 2—plants grown under black net; Group 3—plants grown under blue net.

PCA highlighted that plants grown under sunlight had a positive influence on flavonoid and phenol levels, as well as on leaf (LDW), root (RDW), stem (SDW), total (TDW) dry weight, and total trichome number abaxial (TTNab). Plants grown under black net had a positive influence on variables, such as carotenoids, total chlorophyll, and total trichomes number adaxial (TNTad). The blue net had a strong positive impact on total leaf area (TLA), plant length (PL), and iridoid and total chlorophyll levels. Thus, PCA results corroborate and complete previous analyses and interpretations.

3 Conclusion

Significant differences were observed in *Stachytarpheta sellowiana* growth, development, anatomy, photosynthetic pigment production, and secondary metabolism when plants were grown under different colored nets. Cultivation under sunlight led to the highest dry weight accumulation in plants. Therefore, manipulating the light spectrum by using colored nets can be an effective strategy to promote plant growth and the synthesis of phytochemicals of interest. These results provide a new perspective on the use of blue photosselective shading nets in *Stachytarpheta sellowiana* cultivation to increase antioxidants (TAC) and iridoids' production.

Supplementary Information

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Supplementary Material 1.

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Author contributions

All authors contributed substantially to the work reported. TO, MLG, JEBPP, and SKVB conceived the study, designed the experiments; TO, RMAA, JJFL, TFM and PPB, performed experiments and analyzed data; EA Scanning Electron Microscopy analysis; TO, JPMR and SKVB did the chemical analyses. TO, SKVB, and JEBPP wrote the manuscript. All authors read and approved the manuscript.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate

The collection and experimental use of plant material of *Stachytarpheta sellowiana* Schauer were conducted in accordance with institutional, national, and international guidelines and legislation. Plant material was obtained and handled following the regulations of the Conselho de Gestão do Patrimônio Genético (CGEN) and the Brazilian Law on Access to Genetic Heritage (Law No. 13,123/2015) and its regulatory Decree No. 8,772/2016. The study complied with the principles of the Nagoya Protocol on Access and Benefit-sharing. All experimental procedures were performed at the Universidade Federal de Lavras in accordance with institutional guidelines for research with plant species. No endangered or protected species were involved in this study. This research was registered in the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under registration number A6FD6DA.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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