

Triterpenoid saponins in *Aralia elata* subjected to combined nutrient availability and light quality

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Abstract

Combined light spectra and nitrogen (N) availability may modify contents of triterpenoid saponins (TSs) in leaves of *Aralia elata* (Miq.) Seem. In this study, *A. elata* seedlings were raised under light-emitting diode spectra in red- (26.6% red, 59.9% green, and 13.5% blue) and green-colours (12.6% red, 84.6% green, and 2.9% blue) both at a photosynthetic photon flux density of about 77.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$. N availability was employed at low and high rates of 30 and 90 mg kg^{-1} , respectively. Aralosides-A and -VI did not show any responses to either light or N treatments (ranges of 1.98-3.75 mg g^{-1} and 0.21-1.41 mg g^{-1} , respectively). Compared to the green light, the red light resulted in greater growth but lower foliar N assimilation and aralosides-B ($\sim 0.7 \text{ mg g}^{-1}$) and -V concentrations ($\sim 16 \text{ mg kg}^{-1}$). The high N availability resulted in greater growth, biomass, foliar chlorophyll and protein concentrations but lower N assimilation and TS concentrations. We conclude that araloside B can be taken as an objective TS harvested in *A. elata* food-used leaves as a bioactive compound that can be adjusted by light and N manipulations.

Keywords: *Aralia elata* (Miq.) Seem; araloside; fertility; light quality; triterpene saponins

Introduction

Triterpenoid saponins (TSs) are glycosides consisting of a sugar moiety (glycone) and a triterpenoid component (aglycone). They are increasingly significant due to the functions of countering cancer, tumor, arrhythmia, and inflammation as an efficient and safe ingredient (Du *et al.*, 2014; Han *et al.*, 2021). TSs are in a group of natural products that are considered defensive compounds against biotic and abiotic stressors (Sawai and Saito, 2011). Even factors that are essentially needed by plants may also promote upregulation of TSs when the dose exceeds the range for appropriate growth demand (Cheng *et al.*, 2021a; Gao *et al.*, 2019; Wei *et al.*, 2020c; Wei *et al.*, 2020d). TSs have been found in over 500 plant species across crops, flowers, vegetables,

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weeds, and shrubs (Bartnik and Facey, 2017). Medicinal plants that dwell in the habitat of undergrowth layer are the main group to harbor TS extracts. Accumulation of TSs was found to be abundant in dicotyledonous families, such as Araliaceae (Lavoie *et al.*, 2021; Man *et al.*, 2010; Ponou *et al.*, 2021).

Aralia elata (Miq.) Seem is a shrub with medicinal adaptogen from Araliaceae family (Panossian *et al.*, 2021). Newly grown but unopened buds are termed “Cilaoya” in Chinese as a mark of a native vegetable (Han *et al.*, 2021; Sun *et al.*, 2017a). New buds are also termed “Taranoki” in Japanese for the recognition of a gorgeous vegetable (Kuang *et al.*, 1996; Saito *et al.*, 1990). Vigorous demand for bud harvest leads to heavy exploitation of natural resources from *A. elata* populations, but unordered activities for bud collection have resulted in interruption of natural regeneration (Wei *et al.*, 2019b). Both buds and leaves were found to have enriched concentrations of TSs, which is responsible for enriched reserves of TSs (Sun *et al.*, 2017b; Zhang *et al.*, 2006). The harvest of TSs is a major goal to collect *A. elata* resources. Artificial culture has been suggested to supplement an access to meet heavy demand for harvesting buds and leaves (Cheng *et al.*, 2021b; Gao *et al.*, 2019; Wei *et al.*, 2020c). Even so, natural habitat is still an optimum site for transplant of artificially cultured seedlings and an incubation to promote regeneration (Duan *et al.*, 2022). Natural populations of this species distribute in undisturbed lands of temperate forests dominated by timber trees across montane areas of China, Japan, Korea, and Russia (Wei *et al.*, 2019a; Wei *et al.*, 2021). Tall trees associate with *A. elata* undergrowth in the ecosystem which leads to an adaptive acclimation to shading (Wei *et al.*, 2020c; Wei *et al.*, 2021). This makes natural regeneration more necessary than expected as *A. elata* individuals are frequently subjected to the shading environment in most time of their life spans. Shading is a necessary precondition for the biosynthesis of TSs in *A. elata* leaves (Cheng *et al.*, 2021a; Gao *et al.*, 2019). To figure out the mechanism of TS generation in undergrowth condition is a key approach to rehabilitate regeneration.

Demand for raw *A. elata* mass is increasing by medicinal and edible usages. TSs are the main active ingredients in *A. elata*, which have been characterized by anti-functions to counter tumor, arrhythmia, inflammation, and virus (Han *et al.*, 2021). TSs in *A. elata* leaves have also been found to function in liver protection, blood sugar lowering, blood lipid lowering, and pain relief effects (Luo *et al.*, 2019; Wang *et al.*, 2014). Due to the abundance in extracts of TSs, *A. elata* received intensive research for chemistry and pharmacology since the 1950s (Han *et al.*, 2021). Leaves are one of the major organs in *A. elata* that were taken as the pool of TSs (Cheng *et al.*, 2021a; Han *et al.*, 2021; Lavoie *et al.*, 2021). Leaves are an alternative organ instead of buds that produces dry mass with abundant TSs. Currently, TS compounds that were extracted from *A. elata* included aralosides in types-A, -B, -V, and -VI, ginsenoside R₀ (i.e., chikusetsusaponin V), and chikusetsusaponin Iva (Kuang *et al.*, 1996; Sun *et al.*, 2017a; Xia *et al.*, 2019; Yang *et al.*, 2018). Methodology to determine these TS compounds have been well established (Han *et al.*, 2021; Sun *et al.*, 2017a; Wang *et al.*, 2014). However, the detection of mechanism about the relationship between environmental factors and TS accumulation is still scarce. It is also unclear about the optimum combination of synthesized environmental factors that may best benefit the synthesis and accumulation of TS compounds in *A. elata* leaves. Even more uncertainties exist about the inner relationships between TSs and other foliar physiological variables that may impose relevant effects for TS accumulation in leaves. To determine the effect of ambient factors on TSs is important as the basis to manage and improve the quality of *A. elata* using their leaves.

As a natural product, plant-derived TS is reasonable to be determined by factors that impose effects on plant growth and development. Natural *A. elata* populations dwell in the understory layer of temperate forests, where transmittance through tall tree canopy drives the light condition for undergrowth (Wei *et al.*, 2019a; Wei *et al.*, 2021). Although *A. elata* can tolerate low to moderate shading at intensities around 40–60% of original sunlight, they cannot endure heavy shading as high as ~80% or growth and biomass will be heavily stressed (Cheng *et al.*, 2021a; Gao *et al.*, 2019). In contrast, biosynthesis of TSs in *A. elata* leaves will also be enhanced (Cheng *et al.*, 2021a) in synchronization with the upregulation of protective system for resistance

(Gao *et al.*, 2019). Foliar physiology in *A. elata* saplings of natural populations was also found to vary across forested habitats where light quality in transmittance was different (Wei *et al.*, 2019a). Furthermore, changes in light quality can modify total saponin content in *A. elata* seedlings (Wei *et al.*, 2020c). These together suggest that the difference of light qualities in sunlight spectrum has the potential to change and adjust TSs to the level that we need (Shafiq *et al.*, 2021; Tang and Liesche, 2017; Yu *et al.*, 2020). Relevant information, however, is limited in specifically quantitative TSs in leaves of *A. elata* subjected to more than one spectrum.

Soil nutrient availability is another important factor that can generate an interaction with light spectrum to affect plant growth and physiology (Gao *et al.*, 2021; Li *et al.*, 2018; Zhao *et al.*, 2020). Nitrogen (N) is an essential element that is needed by plants for growth and dry mass production. Appropriate N availability can benefit TS synthesis in medicinal plants from Araliaceae family (Qin *et al.*, 2020; Wei *et al.*, 2020d; Zhang *et al.*, 2020). For example, N application at appropriate rates can promote the synthesis of saponins through improving root growth and N uptake in *Panax notoginseng* (Wei *et al.*, 2020c). Zhang *et al.* (2020) concluded that appropriate N availability benefits TS biosynthesis through promoting accumulation of carbon (C) based metabolites from the interaction between N and phytohormones in processing N acquisition where TS might be derived in *P. notoginseng*. To our knowledge, quite less has been known about the effect of N availability on TS in *A. elata*.

Foliar N concentration in *A. elata* has been detected as a predictor to indicate acclimation to ambient understory conditions (Wei *et al.*, 2019a; Wei *et al.*, 2019b). Pilot study also revealed that the N addition had an interaction with light quality to affect N uptake and utilization in *A. elata* (Wei *et al.*, 2020a). These findings together suggest a probability that light spectrum and N availability might generate an interaction to cause the difference of TSs in *A. elata* in natural populations. To detect this interaction will supply further evidence to collect TSs from natural populations of *A. elata* with a full employment of artificial lighting on N availability and higher production of TSs.

In this study, *A. elata* was cultured as seedlings which were subjected to contrasting conditions of light spectra and N availabilities. Light quality and N doses were determined by simulating from investigations in natural populations. Our objective was to detect the interactive effects of light quality and N availability on TSs and their associations with seedling parameters in *A. elata*. Based on current evidence, we hypothesized that TSs concentration would be increased in conditions where combined light and N availability stressed *A. elata* growth.

Materials and Methods

Plant materials and growth condition

This study was carried out in the Laboratory of Combined Manipulation of Illumination and Fertility on Plant Growth (43°58' N, 125°24' E). Seeds were collected from mature *A. elata* plants in a population at remote rural area of eastern Harbin city (45°27'-46°04' N, 129°00'-129°40' E), Heilongjiang province, Northeast China (Wei *et al.*, 2019b). We chose this place for seed collection because it has been demonstrated that mature plants that distributed on 9°-slope tended to have balanced N and phosphorus (P) reserves with high nutrient availability which unlikely generated uncontrolled influence on future results due to lack of nutrients. Seeds were kept in wet sands 1.5 m belowground for two years then they were excavated in fall and sowed in water for 48 h. Seeds were sterilized by soaking in 0.5% (w/w) potassium permanganate for 30 min. Subsequently, they were sown in commercial substrates for germination. Substrates were generated as a mixture of peat, spent mushroom residues, and perlite in proportional ratios of 55:25:20 (v/v/v) (Mushro-Dust, Zhiluntuowei A&F S&T Inc., Changchun, China). This substrate has been used in studies on various types of plants with an appropriate response in growth and nutrient uptake at the initial growing stage (Li *et*

al., 2021b). Germinated seedlings were incubated in growing-trays with plugs in height of 13 cm and top-diameter of 7 cm. This type of trays can facilitate rooting to a deep depth when seedlings were prepared to be transplanted (Luo *et al.*, 2020; Wei *et al.*, 2020c; Xu *et al.*, 2019).

Germinated seedlings were used for further culture with a uniform size as about 5.00 ± 0.30 cm in height and 0.40 ± 0.10 cm in root-collar diameter (RCD). This was chosen as they met the standard of initial sizes of *A. elata* seedlings in successful cases (Wei *et al.*, 2019a; Wei *et al.*, 2020a). Seedlings were transplanted to plastic 0.68 L-pots (11.5 cm \times 7.5 cm \times 9.5 cm, top diameter \times bottom diameter \times height) that were filled with purified perlites. This type of pots have been used in studies on undergrowth with unlimited root system development (Gao *et al.*, 2021; He *et al.*, 2021; Tan *et al.*, 2021; Zhou *et al.*, 2022). Eight pots were placed in a plastic tray (55 cm \times 35 cm, length \times width) where water was maintained at a depth of about 3 cm to enable sub-irrigation (Liu *et al.*, 2021; Wei *et al.*, 2017; Zhao *et al.*, 2019). During the experiment, temperature was controlled in a range of 19–28 °C (night/day) with an averaged relative humidity (RH) of $83 \pm 6\%$.

The treatment of light quality control

Throughout the experiment, no sunlight was allowed to illuminate at *A. elata* seedlings by lighttight curtains beyond windows. All lights were supplied by light-emitting diodes (LEDs) by panels in an area of 0.48 m² (1.2 m \times 0.4 m, length \times width). LED panels were hanged over seedlings with lights downwards. Electrical current of diodes emitting lights enriched in red (600-700 nm), green (500-600 nm), and blue (400-500 nm) colours were managed by transformers. Electricity for red-light diodes was controlled by a 200-W transformer and that those for green- and blue-lights by a 150-W transformer. Therefore, meanwhile adjustments of electrical currents of diodes for three colours of lights will enable the change of lighting quality.

We aimed to create contrasting lighting conditions with different spectra but at a similar illumination intensity. This design has been taken as a scientific approach to test varied effects of lighting spectra on performance of objective plant species (Li *et al.*, 2018; Luo *et al.*, 2020; Zhao *et al.*, 2019; Zhao *et al.*, 2020). We determined parameters of lighting conditions by simulating sunlight quality in transmittance of natural *A. elata* habitats.

Natural *A. elata* populations dwell in six forest types in mountains at Northeast China (Wei *et al.*, 2019a; Wei *et al.*, 2021). Therein, the relative ratio of photosynthetic photon flux density (PPFD) in green light to that in red light (G/R) was found to be a crucial variable whose change will diversify TSs contents in *A. elata* seedlings (Wei *et al.*, 2020c). Therefore, we employed two different spectra with contrasting green to red light ratios of 2.25 and 6.77 for red- and green-colour spectra, respectively (Table 1). The red colour spectrum was achieved by adjusting electrical currents for diodes in red, green, and blue colours to be 20%, 10%, and 30% of the full power, respectively; while the green colour spectrum to be 20%, 100%, and 10%, respectively. Specific wavelengths for the two types of absolute spectra can be seen in Figure 1.

Table 1. Simulated optical variables in contrasting light-emitting diode (LED) spectra for *Aralia elata* seedlings

Spectra	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Red (%)	Green (%)	Blue (%)	G/R
Red colour	77.59	26.6	59.9	13.5	2.25
Green colour	77.37	12.5	84.6	2.9	6.77
Mean	77.48	19.55	72.25	8.20	4.51
SE	0.11	6.81	11.93	5.12	2.18

All values were measured 40 cm beneath the LED panel. G/R, green to red light ratio. SE, standard error.

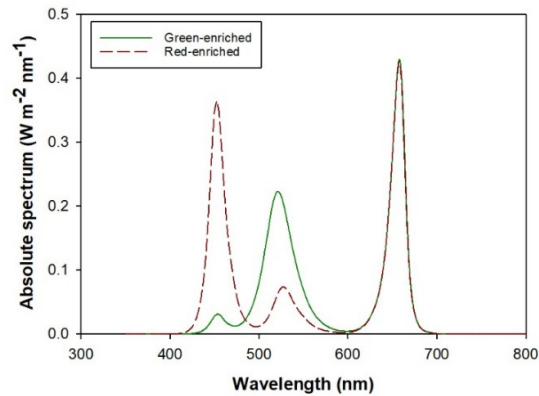


Figure 1. Absolute spectrum values in wavelengths of green-enriched and red-enriched lights

In an investigation on a total of 34 *A. elata* populations across forests in Heilongjiang and Jilin provinces at Northeast China, instant PPFD of sunlight was measured to be an average of $528.33 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the open-air beyond canopies over *A. elata* populations (Wei *et al.*, 2019a). The average ratio of PPFD under canopy to that without shading was averaged to be 14.67%. Therefore, most *A. elata* individuals were subjected to a transmittance with PPFD of $77.48 \mu\text{mol m}^{-2} \text{s}^{-1}$. To simulate the similar level of this dose for two types of spectra, the LED panels were always kept a length of 40 cm above seedling shoot-tips throughout the experiment.

The treatment of nitrogen availability

Again, during the investigation on a total of 34 natural *A. elata* populations, it was found that in the stand (20 m × 20 m) located at Wuying county, Heilongjiang province, soils exhibited lowest level of N availability, while another stand at Yichun city showed the highest level (Wei *et al.*, 2021). In the stand at Wuying county, ammonium N (AN) and nitrate N concentrations (NN) were measured to be 26.73 mg kg^{-1} and 3.83 mg kg^{-1} , respectively. These together led to a total of mineral N concentration of 30.56 mg kg^{-1} . In Yichun stand, AN and NN were measured to be 86.46 mg kg^{-1} and 3.58 mg kg^{-1} ; hence total mineral N was 90.04 mg kg^{-1} . Therefore, half of *A. elata* seedlings were subjected to N addition at a rate of 30 mg kg^{-1} (w/w) to mimic the low N availability, while the other half received the rate of 90 mg kg^{-1} (w/w) to mimic the high availability. All N additions were delivered through NH_4NO_3 solutions with P added at a rate of 45 mg kg^{-1} and potassium (K) at 60 mg kg^{-1} for seedlings. Nutritional solutions were pulsed to the surface of perlite substrates to avoid N contamination of aerial organs (Gao *et al.*, 2021). A volume of 10 mL nutritional solutions was applied using a 5 mL pipettor twice a week from the time of one week after transplant to the end of experiment.

Experimental design and arrangement

This study was carried out as a split-block design with two light spectra as the main plots and two levels of N availabilities nested as sub-plots. The random placement of trayed pots of seedlings receiving both N availabilities in one spectrum was taken as the random block that accounted for errors in the combination between main plot and random group. One tray with eight pots of seedlings (each pot with one seedling) was assigned as one basic measuring unit and three blocks were assigned as three replicates of units. All pots and trays were rearranged for their places every time after N pulsing to eliminate edge effect.

Seedlings were commenced to be transplant at mid-April of 2018. In the first week after transplant, seedlings were subjected to sub-irrigation with distilled water without additional N applied. Applications of N were delivered for four months up to late August. One week after the last application of nutritional solution pulse, seedlings were sampled for further determination

Seedling sampling and measurements

Eight pots of seedlings were sampled per tray, and all trayed seedlings were measured for height and RCD. Thereafter, four of them were used for measuring dry mass weight and all subsequent variables using dried samples. The other four were used for determining variables that needed fresh samples. The first four seedlings were divided into shoot and root parts, and both were dried in an oven at 65 °C for 72 h. Dry mass biomass was measured for both types of parts. Dried samples were collected and mixed for all four seedlings per tray. These dried mixtures will be used for determining N concentration in shoot and root parts using the Kjeldahl method.

Determination of chlorophyll and protein contents

The other four seedlings per tray were washed by distilled water then leaves were excised off and freeze-dried. Leaf samples were ground in a mill at a screen of 0.42 mm. Chlorophyll-a and -b and carotenoid were determined by 65 °C bathing in dimethyl sulfoxide and measured using spectrophotometer at 663 nm, 645 nm, and 470 nm, respectively, using the following formulations (Li, 2000):

$$C_a = 12.21 \times A_{663} - 2.81 \times A_{645} \quad (1)$$

$$C_b = 20.13 \times A_{645} - 5.03 \times A_{663} \quad (2)$$

$$C_{caro} = \frac{1000 \times A_{470} - 3.27 \times C_a - 104 \times C_b}{229} \quad (3)$$

where, C_a , C_b , and C_{caro} are concentrations for chlorophyll-a, chlorophyll-b, and carotenoid; A_{663} , A_{645} , and A_{470} are absorbances at 663 nm, 645 nm, and 470 nm, respectively.

Soluble protein was extracted for three times with 80% (v/v) ethanol at 80 °C in water bathing for 5 min. Samples were centrifugated for 5 min at 1300× rpm and moved to a test tube with phosphate buffer (0.2 M, pH 4.9). Solution was centrifuged again and the supernatants were collected with Coomassie Blue reagent by a spectrophotometer at absorbance of 595 nm (Bradford, 1976).

Assessment of leaf glutamine synthetase activity

Leaf glutamine synthetase (GS) activity was determined according to a method of Yu *et al.* (2012) with minor modifications. Samples in weight of half gram were suspended in 5 mL extraction buffer solution. The buffer was made by 50 mM Tris-HCl (pH 8.0), 2 mM MgSO₄, 0.4 M sucrose, 2 mM dithiothreitol, 5% (w/w) polyvinylpyrrolidone, and homogenized in a chilled mortar (by liquid N) and pestle. The homogenate was centrifuged at 8,000× rpm for 30 min at 4 °C. An aliquot in the volume of 0.7 mL was collected from the extract and mixed with 1.6 mL of a reacting mix (100 mM Tris-HCl, 80 mM MgSO₄, 20 mM sodium glutamate, 80 mM NH₂OH, 20 mM cysteine, 2 mM ethylene glycol tetraacetic acid, pH 7.4) and 0.7 mL of 40 mM ATP solution. After reaction at 37 °C for 20 min, a volume of 1 mL stop-solution (0.37 M FeCl₃, 0.2 M trichloroacetic acid and 0.6 M HCl) was added to terminate the reaction. The supernatant was centrifuged again at 8,000× rpm for 30 min at 4 °C and analysed at the absorption of 540 nm. GS activity was assessed as μmols of γ-glutamyl hydroxamate formed per mg-weight of protein in one hour.

Determination of leaf triterpenoid saponin

Concentrations of TSs in *A. elata* leaves were determined by adapted methods by Kuang *et al.* (1996) (araloside A and araloside B), Xia *et al.* (2019), and Sun *et al.* (2017a) (araloside V and araloside VI). A total of 10 mg freeze-dried samples were ground and dissolved to 2 mL of 70% methanol (v/v). Solutions were incubated ultrasonically at 28 kHz and 80 °C for 30 min. Supernatants were centrifuged at 12,000× rpm for 10 min, cooled to room temperature, and received 50% ethanol (v/v) to compensate for the weight loss. Extracts were filtrated through a 0.22 μm filter, dried in vacuum, and used for quantification in 5 μL. Analytical quantification was achieved by an ultra-performance liquid chromatography (UPLC) electrospray ionization-

tandem mass spectrometry in multiple reactions monitoring (MRM) mode (UPLC-MRM-MS) system. A Waters Acquity UPLC H-Class system was equipped with a sample management system (Waters Inc., Milford, MA, USA), which were together used for liquid chromatography. UPLC separation was achieved on a high strength silica (HSS) T3 column (2.1×150 nm, $1.8 \mu\text{m}$) coupled with a Waters HSS T₃ guard column (2.1×5 mm, $1.8 \mu\text{m}$) at 35°C in columns. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid acetonitrile. The UPLC condition was optimized for *A. elata* leaves as following gradient elution (Xia *et al.*, 2019): 0–1.5 min, 10%-35% B; 1.5–7.0 min, 35-35% B; 7.0–7.5 min, 35-50% B; 7.5–11.0 min, 50-90% B; 11.0–11.5 min, 90-90% B; 11.50–11.51 min, 90%-10% B; 11.51–16.00 min, 10%-10% B. Typical ion current chromatograms for standard reference and samples are shown in Figure 2.

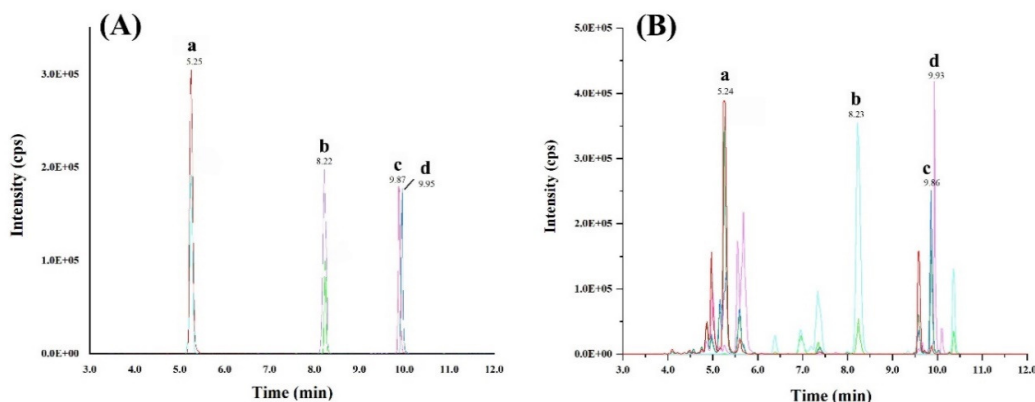


Figure 2. Ion current chromatograms in standard reference (A) and *Aralia elata* sample (B) identified by an ultra-performance liquid chromatography electrospray ionization-tandem mass spectrometry in multiple reactions monitoring mode system. Component peak interpretations: a, araloside VI; b, araloside araloside VI; c, araloside A; d, araloside B

Statistical analysis

Data were analysed by SAS software (SAS Institute, Cary, UC, USA). Data were analysed by a split-block design with contrasting light qualities as the main plot and nutrient availabilities as the sub-main plot. Each combined treatment had three replicates. Spectra and nutrient effects were tested by two-way analysis of variance (ANOVA). When significant effects were indicated, results were compared and arranged by means in according to Tukey test ($\alpha=0.05$). Principle component analysis (PCA) was employed to test data variation for variables and trends of inner relationships among variables (Liu *et al.*, 2021; Wang *et al.*, 2020).

Results

Growth and biomass accumulation

Both light spectra and N availability had a main effect on shoot height, RCD, and biomass in shoot and root, but no interactive effects were detectable (Table 2). Both height and RCD were higher in the red spectrum than in the green spectrum by 21.5% and 16.7%, respectively (Figure 3A, C). These two variables were also higher in the higher N availability. Compared to the low N availability, seedling height and RCD increased by 14.9% and 19.0% in high N availability, respectively (Figure 3B, D).

Table 2. *F* values from analysis of variance (ANOVA) of light spectra (L), nutrient availability (N), and their interaction (L × N) on variables in growth, biomass, photosynthetic pigment, foliar nitrogen (N) concentration and assimilation, and secondary metabolisms in *Aralia elata*

Variable	L	N	L × N
Shoot height	12.35**	6.31*	0.14
RCD	8.80*	11.18*	0.50
Shoot biomass	13.94**	25.70**	2.21
Root biomass	20.27**	9.84*	0.38
Chlorophyll-a	4.32	10.61*	0.98
Chlorophyll-b	0.65	19.69**	5.08
Carotenoid	1.99	10.88*	0.34
Protein	2.05	5.52*	0.50
Shoot N concentration	9.36*	46.36**	31.48**
Root N concentration	8.36*	10.35*	7.47*
GS activity	16.71**	9.92*	1.22
Araloside A	1.64	2.08	0.02
Araloside B	6.79*	10.09*	0.01
Araloside V	17.28*	13.76*	0.07
Araloside VI	5.15	4.65	1.17

Asterisks indicate significance of results: *, $P < 0.05$; **, $P < 0.01$; GS, glutamine synthetase.

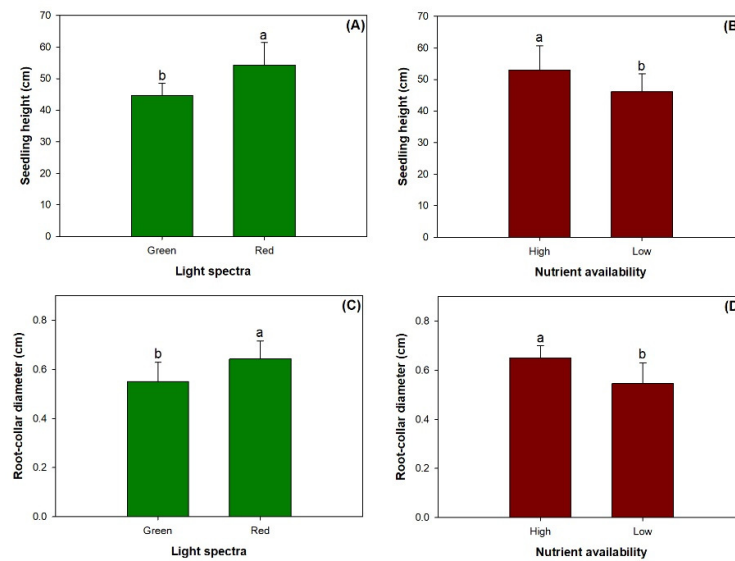


Figure 3. Shoot growth of *Aralia elata* seedlings cultured in high and low nutrient availabilities under green-enriched and red-enriched light spectra. Columns stand for mean values of seedling height (A and B) and root-collar diameter (C and D) with error bars as standard deviations. Different letters indicate significant difference according to Tukey test at 0.05 level

Light spectra and N availability had no interactive effects on biomass in shoot and root of *A. elata* seedlings (Table 2). Instead, either light spectra or N availability had a significant main effect on biomass. Compared to the green-light enriched spectrum, the red-light spectrum resulted in greater biomass in shoot and root by 66.7% and 56.3%, respectively (Figure 4A). Compared to low N availability, the high availability increased biomass in shoot and root by 102.8% and 36.1%, respectively (Figure 4B). In addition, neither treatment had significant effect on root to shoot biomass ratio (R/S) (light, $F=0.31$, $P=0.5903$; N, $F=4.63$,

$P=0.0636$). The R/S value ranged 0.17 and 0.19 in response to contrasting light spectra, and it ranged in 0.14-0.22 in response to different N availabilities.

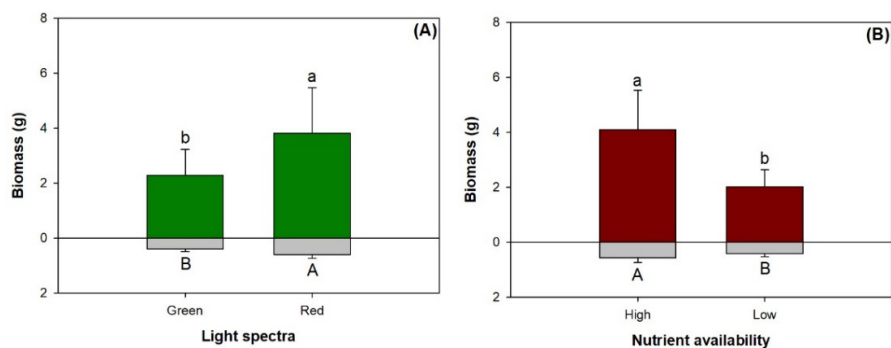


Figure 4. Biomass accumulation in *Aralia elata* seedlings cultured under green-enriched and red-enriched light spectra (A) in high and low nutrient availabilities (B). Columns stand for mean values of biomass in shoot (green and red colours) and root (grey colour) with error bars as standard deviations. Different letters indicate significant difference according to Tukey test at 0.05 level. Lower-case letters a and b mark significant difference for shoot, and capital letters mark difference for root

Photosynthetic pigment and protein

Light spectra and N availability had no interactive effects on foliar chlorophyll and carotenoid concentrations (Table 2). In addition, light spectra had no effect on photosynthetic pigments, but the treatment of N availability was effective. High N availability resulted in higher concentrations in chlorophyll-a, chlorophyll-b, and carotenoid by 135.7%, 85.6%, and 221.5% compared to low N availability, respectively (Figure 5A-C). High N availability also led to higher soluble protein concentration by 6.5% compared to low N availability (Figure 5D).

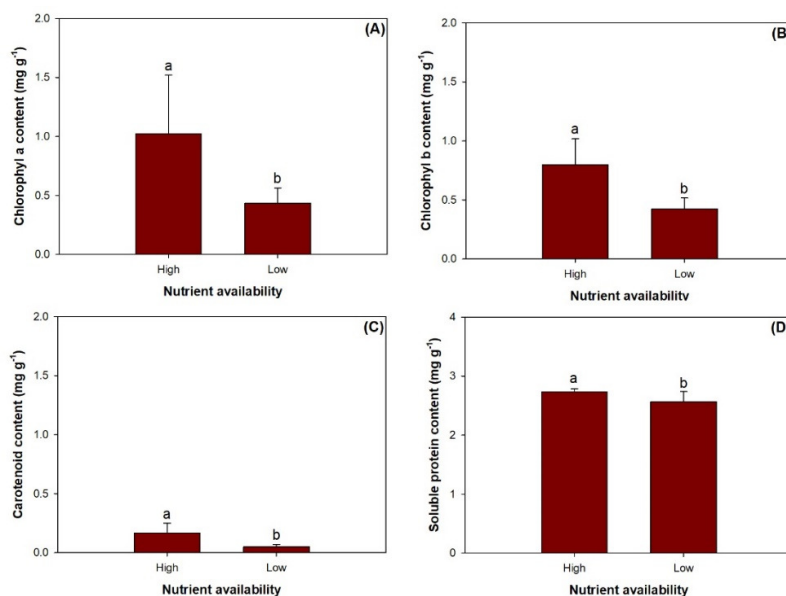


Figure 5. Foliar chlorophyll-a (A), chlorophyll-b (B), carotenoid (C), and soluble protein (D) concentrations in *Aralia elata* seedlings cultured in high and low nutrient availabilities. Columns stand for mean values with error bars as standard deviations. Different letters indicate significant difference according to Tukey test at 0.05 level

N concentration and assimilation

Light spectra and N availability had interactive effects on N concentration in shoot and root of *A. elata* seedlings (Table 2). Seedlings exposed to low N availability in the green-light enriched spectrum had highest N concentration in both shoot and root parts among all combined treatments (Figure 6A). In shoots, low N availability in the green-light enriched spectrum resulted in higher N concentration by 26.3-42.5% compared to that in other treatments. In roots, N concentration in low-N and green-light spectrum was higher than that in other treatments by 21.0-22.5%.

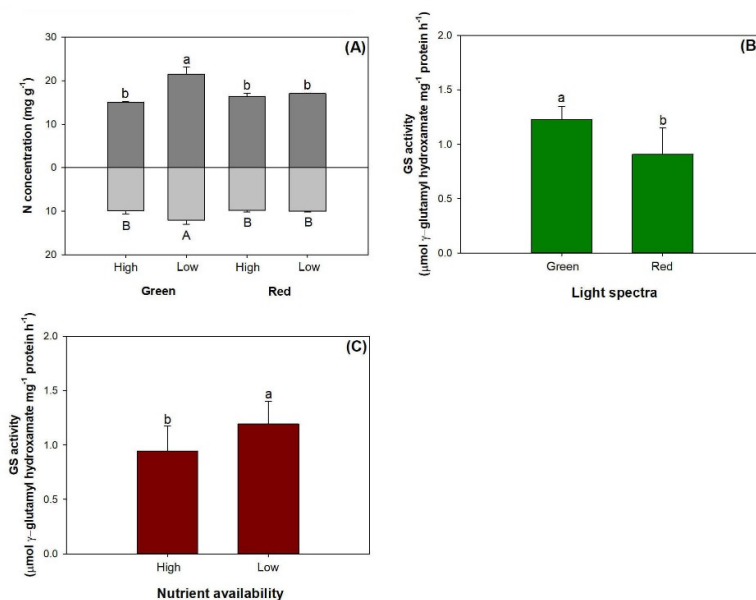


Figure 6. Shoot and root nitrogen (N) concentration (A) and foliar glutamine synthetase (GS) activity (B and C) in *Aralia elata* seedlings cultured in high and low nutrient availabilities under green-light and red-light enriched spectra. Columns stand for mean values with error bars as standard deviations. Different letters indicate significant difference according to Tukey test at 0.05 level. In (A), lower-case letters remark significant difference in shoot (dark grey), capital letters remark significant difference in root (light grey)

Either light spectra or N availability had a main effect on GS activity (Table 2). Compared to the red-light spectrum, the green-light spectrum resulted in higher GS activity by 35.5% (Figure 6B). Compared to high N availability, low N availability resulted in higher GS activity by 26.3% (Figure 6C).

Triterpenoid saponin concentration

Light spectra and N availability had no interactive effects on aralosides-A, -B, -V, and -VI concentrations in leaves (Table 2). Either of factors also had no effects on aralosides-A and -VI concentrations. Araloside A concentration ranged between 1.98 mg g⁻¹ and 3.75 mg g⁻¹. Araloside VI concentration ranged between 0.21 mg g⁻¹ and 1.41 mg g⁻¹. However, both light spectra and N availability had a main effect on aralosides-B and -V concentrations (Figure 7). Aralosides-B and -V concentrations were higher in the green-light enriched spectrum by 68.3% and 138.3% in the red-light enriched spectrum, respectively (Figure 7A, C). Compared to aralosides-B and -V concentrations in the high N availability, those in the low N availability increased by 90.1% and 114.9%, respectively (Figure 7B, D).

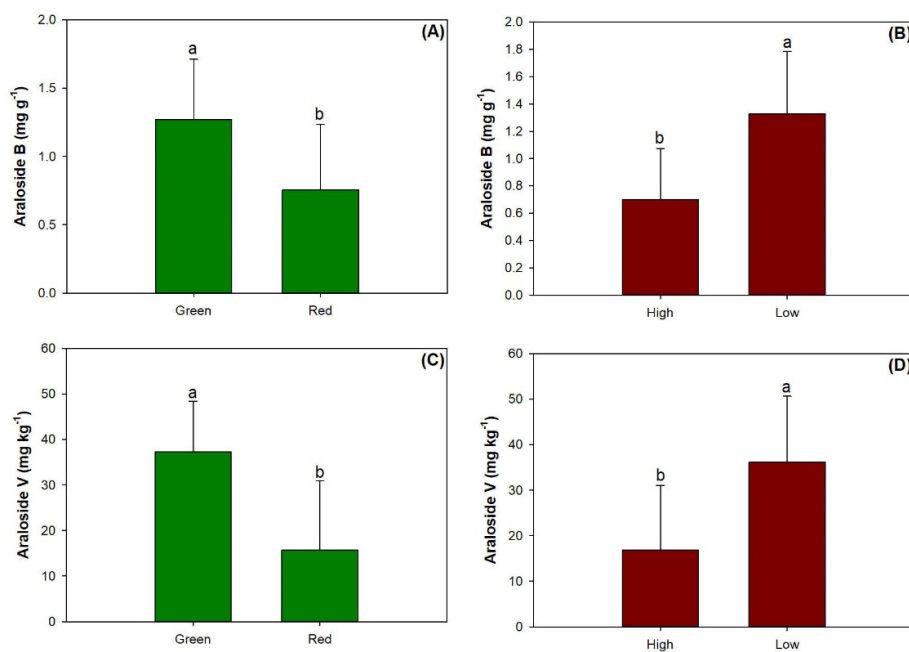


Figure 7. Foliar araloside B (A and B) and araloside V concentrations (C and D) in *Aralia elata* seedlings cultured under green-light and red-light enriched spectra (left) in high and low nutrient availabilities (right). Columns stand for mean values with error bars as standard deviations. Different letters indicate significant difference according to Tukey test at 0.05 level

Principal component analysis

The first two principal components (PCs) account for 70.13% of cumulative eigenvalues, wherein the first PC accounts for 48.47% of data variation and the second for 21.66% (Figure 8). All four TS concentrations showed a crowded data variation which together showed a positive relationship with N concentration and a negative relationship with shoot growth (height and RCD) and biomass accumulation. Although GS showed no relationships with N concentration or TS concentrations, GS showed a negative relationship with foliar chlorophyll-a and protein concentrations. In addition, foliar N concentration also showed a negative relationship with chlorophyll-b and carotenoid concentrations.

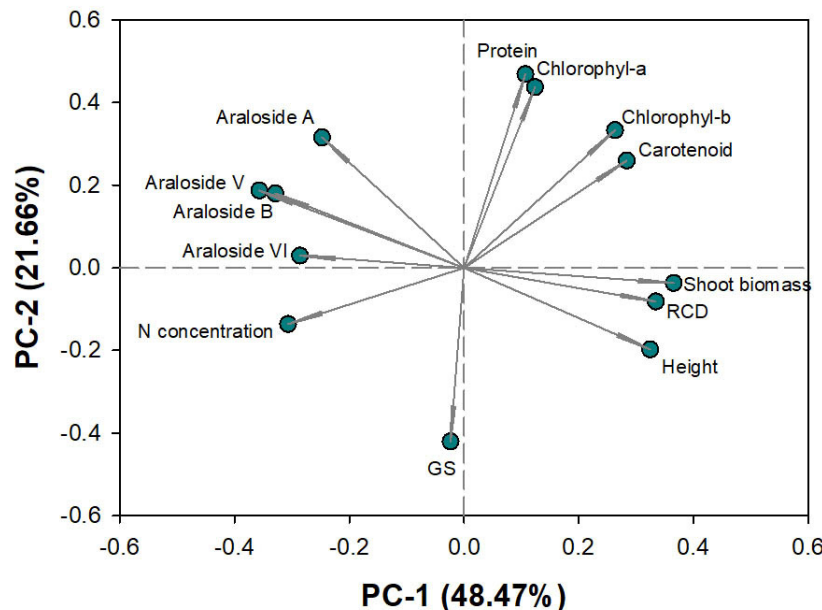


Figure 8. Principal component analysis (PCA) of variables about growth, biomass accumulation, foliar physiology, nitrogen (N) uptake, and triterpenoid saponin (TS) concentration in *Aralia elata* seedlings cultured under green-light and red-light enriched spectra in high and low nutrient availabilities. The x-axis represents the first PC eigenvalues and y-axis for the second. Abbreviations: Protein, foliar protein concentration; chlorophyll-a and -b, foliar concentrations of chlorophyll-a and -b; carotenoid, foliar carotenoid concentration; RCD, root-collar diameter; GS, glutamine synthetase; araloside A, araloside B, araloside V, araloside VI, foliar concentrations of these four TSs

Discussion

We found araloside A concentration ranged between 1.98 mg g^{-1} and 3.75 mg g^{-1} in leaves. This dose was much lower than that in root bark of *Aralia elata*, which ranged between 50 and 100 mg kg^{-1} (Lee *et al.*, 2005). Our araloside VI concentration ranged between 0.21 mg g^{-1} and 1.41 mg g^{-1} (equally 0.02 - 0.14%), which were lower than that (0.14 - 0.72%) reported by (Sun *et al.*, 2017a). These lower records of our samples compared to those in previous studies were resulted from the differences of material sources. Firstly, root bark contained more abundance of TSs than leaves in *A. elata*. However, root barks do not have a potential to be used as a source of food as expected as leaves. Secondly, our leaf araloside VI concentration was lower because our plants were cultured in an all-controlled environment, where light and nutrient conditions were simulated from natural settings. Leaves in Sun *et al.* (2017a) were all collected from natural populations or field cultivars, which had a longer age and more sufficient accumulation of natural products. The understory condition where natural *A. elata* dwells in is determined by joint syntheses of forest structure (Wei *et al.*, 2021), topography (Wei *et al.*, 2019b), and sunlight transmittance (Wei *et al.*, 2019a; Wei *et al.*, 2020a; Wei *et al.*, 2020c). All these conditions were largely different from those controlled in the laboratory where our seedlings were raised. Anyway, leaf araloside-A and-VI concentrations varied in different locations (Sun *et al.*, 2017a), but failed to respond to either light spectra or N availability in our study. These results together suggest that light and N were not key drivers that imposed determinative effects on accumulations of these two TSs in leaves of *A. elata* seedlings. To our knowledge, rare data can be referred to with ours about leaf araloside B concentration. However, our araloside V concentration, ranged of 15 - 37 mg kg^{-1} which was also lower than that in Sun *et al.* (2017a). Both araloside-B and araloside-V concentrations can be modified by either light spectra or N availability. Overall, we

recommend araloside B as an objective TC that can be the extracted bioactive compound from edible leaves of *A. elata* subjected to manually controlled light and N conditions.

It was unexpected that light spectra did not generate any interactive effects with N availability for most of variables except for N concentration. In contrast to our results, light and N availability were found to generate an interaction on tree seedlings (Guo *et al.*, 2022). In accordance to our results, large scale of null response to the interaction between spectra and nutrient availability was also found on *Pinus koraiensis* (Zhao *et al.*, 2020) and *Quercus variabilis* (Gao *et al.*, 2021) seedlings. In contrast, studies on *Larix principis-rupprechtii* (Zhao *et al.*, 2019), *Podocarpus macrophyllus* (Song *et al.*, 2022), and *Eleutherococcus senticosus* seedlings (Guo *et al.*, 2022) revealed frequent interactions for growth and biomass variables. It appears that slowly growing species were difficult to show apparent performance when exposed to light and nutrient interactions, and fast-growing ones will be more reactive. We do not have specific evidence to support this speculation because there is no clear rule to define growing speed across species. In another study where *A. elata* seedlings were tested, height and root biomass were responsive to the interaction (Wei *et al.*, 2020a). The cultural condition therein employed a plant factory environment, where lighting diodes were delivered in growth-promoting spectra and N availability was imposed for intensive loading. As we employed a simulative spectra-N condition, interactions may not be so effective at low levels of influences. As independent driving factors, either light or N availability determined shoot growth and biomass, which further generated negative correlations with leaf TSs. These changes can be used as a prediction of TSs in *A. elata* leaves.

Light spectrum had a strong influence on growth, biomass, N assimilation, and TS accumulation in *A. elata* seedlings. Compared to the green-light enriched spectrum, that enriched in red-light generally induced an enhancement on shoot growth and whole-plant biomass accumulation. Red-light enriched spectrum simulating transmittance was also reported to increase stem length of *A. elata* seedlings (Wei *et al.*, 2020c), although this effect sometimes fail to occur or even generated negative contribution to diameter growth (Wei *et al.*, 2020a). However, more evidence supported our findings by studies on undergrowth plants with better growth and biomass performances in red-enriched spectra (Li *et al.*, 2018; Wang *et al.*, 2020). By contrast, the red-light enriched spectrum depressed N uptake and assimilation by leaves, and it further decreased concentration of TSs in leaves. However, N change had rare relationship with TSs in leaves. Decline of N concentration with promoted growth has been popular in tree seedlings exposed to changed spectra. This was basically ruled by the natural trade-off between dry mass and N reserve during tree seedling growth (Wei *et al.*, 2013; Wei *et al.*, 2017). Manipulation of lighting spectra that promoted growth will, in contrast, strengthen the decline of N concentration (Li *et al.*, 2018; Wei *et al.*, 2020b; Zhao *et al.*, 2019). Depressed N assimilation as decreased GS activity was also reported on *Quercus variabilis* (Gao *et al.*, 2021) and *Pinus koraiensis* (Wei *et al.*, 2020b) seedlings. These results, plus findings in this study, together demonstrated that red-light enriched spectrum can limit N assimilation through glutamine synthesis. In addition, red-light enriched spectrum led to a decrease of TS concentration in *A. elata* leaves relative to more types of natural spectra (Wei *et al.*, 2020c). Studies on *A. elata* also revealed that foliar TS concentration was depressed by heavy shading (Cheng *et al.*, 2021a; Cheng *et al.*, 2021b), suggesting that TS synthesis will depend on a proper illumination regime. Red-light enriched spectrum may mean a stress for TS synthesis in *A. elata* leaves relative to other light spectra. More work is needed to test the stressful response of *A. elata* to different types of light spectra. Thus, we can partly accept our hypothesis according to results about spectra on TS concentration.

In our study, a high N availability, i.e. 90 mg N kg⁻¹, can benefit growth, biomass, and foliar physiology and protein content relative to a low N availability of 30 mg N kg⁻¹. In contrast, higher N availability decreased N uptake and assimilation and TS concentration. As responses of biomass (growth) and N uptake in woody plant seedlings depend on the level of N availability (Xu *et al.*, 2019). Increase in biomass and growth but decrease of N concentration can be caused by over-dose-N induced dilution or depletion of reserved N by remobilization (Duan *et al.*, 2013; Li *et al.*, 2018). In natural population, foliar GS activity in *A. elata*

individuals tended to be enhanced when site condition for soil N availability was improved (Wei *et al.*, 2019b). Improvement of N availability was also an impetus to promote GS activity in oak (Gao *et al.*, 2021) and *Dalbergia odorifera* seedlings (Li *et al.*, 2021a). Hence, we surmise that our high N availability formed a stress for *A. elata* which impaired N assimilation and TS synthesis although seedling size was enlarged. This disagrees to the viewpoint that secondary metabolism can be upregulated to cope with stress (Sawai and Saito, 2011). The synchronization of N and TS in *A. elata* suggests that N acts as some important role in TS synthesis, which needs further work to confirm. Therefore, we can fully accept our hypothesis according to results about both spectra and N availability on TS concentration.

Conclusions

In this study, we cultured *A. elata* seedlings using contrasting light spectra and N availabilities in a controlled environment where levels of manipulating factors were adapted from natural condition. We did not find significant interactive effects between spectra and N availability on most of variables about growth, biomass accumulation, foliar physiology, and TS concentration except for N concentration. However, either of the two factors generated a main effect on *A. elata*. The green-light enriched spectrum and low N availability (30 mg kg^{-1}) resulted in contrasting performance in growth and N assimilation but meanwhile promoted TS concentrations. Araloside-B and -V were the two types of TSs that responded to manipulations. We conclude that TS concentration has a positive relationship with N assimilation, both of which may not always perform as expected as growth performance facing varied light spectra and N availabilities. When leaves are taken as a food material from *A. elata*, araloside B can be recommended as an objective bioactive compound that can have responses at an acceptable range of concentration.

Authors' Contributions

Conceptualization, YS and SG; Data curation, HQ and LT; Formal analysis, YS and LT; Funding acquisition, SG; Investigation, SG and LT; Methodology, SG and LT; Project administration, SG; Resources, HQ; Software, HQ; Supervision, YS; Validation, SG; Visualization, YS and HQ; Writing - original draft, YS; Writing - review and editing, SG. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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