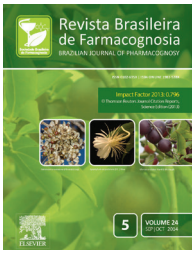
Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.journals.elsevier.com/revista-brasileira-de-farmacognosia



Original article

# Light affects *Varronia curassavica* essential oil yield by increasing trichomes frequency

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ARTICLE INFO

*Article history:*
Received 26 July 2014
Accepted 26 August 2014

*Keywords:*
*Cordia verbenacea*
Irradiance
Medicinal plant
Caryophyllene oxide

ABSTRACT

Light can act on essential oil yield directly on synthesis of secondary metabolites, or indirectly on plant growth. *Varronia curassavica* Jacq., Boraginaceae, is a native medicinal species from Brazil known as "erva-baleeira", with anti-inflammatory activity related to its essential oil. Despite pharmacological evidences of this species and its economic importance for herbal medicine production, little is known about the effect of light on growth and essential oil production. This study aimed to analyze the influence of different irradiances on growth, frequency of trichomes, essential oil yield and composition of *V. curassavica*. The irradiance affected plant growth, but no significant alteration on leaf biomass was detected. The increase in essential oil content under higher irradiance reflected on essential oil yield, and is associated with higher frequency of glandular, globular trichomes. The essential oil composition, rich in caryophyllene derivatives was affected by irradiance, but α-humulene, the constituent of pharmaceutical interest, remained unchanged.


## Introduction

*Varronia curassavica* Jacq. (sin. = *Cordia verbenacea* DC.), Boraginaceae, popularly known as "erva-baleeira", "catinga-de-barão", "maria-preta" and "maria-milagrosa" occurs naturally from Central to South America, and in Brazil is associated with the Atlantic Forest. It is a medicinal plant traditionally used to treat inflammation, ulcers, arthritis, and pain. Phytochemical studies revealed the presence of flavonoids, phenols and essential oils, responsible for its anti-inflammatory, antimicrobial and allergenic activities (Carvalho et al., 2004; Sertié et al., 2005; Ticli et al., 2005; Passos et al., 2007). The main components of *V. curassavica* essential oil are α-pinene (29.69%) and *trans*-caryophyllene (25.27%) other components in significant concentration are *allo*-aromadendreno (9.99%) and α-humulene (4.64%) (Carvalho et al., 2004).

The synthesis, storage and release of essential oils in plants occur in specialized secretory structures, such as oil cells, ducts, lysigenous and schizolysigenous cavities, or glandular trichomes according to its the botanical families (Simões and Spitzer, 2000). In *V. curassavica* the essential oil is secreted and stored in glandular globular trichomes present on the leaf surface (Ventrella and Marinho, 2008), whose frequency and development can be influenced by irradiance (Gomes et al., 2009; Costa et al., 2010).

Besides genetic characteristics, many environmental factors can lead to variations in secondary metabolites content. The monoterpenoids and sesquiterpenoids are metabolites in essential oils often subject to variations due to abiotic factors.

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0102-695X/$ - see front matter 
http://dx.doi.org/10.1016/j.bjp.2014.10.005

The light intensity can change the essential oil production via activation of photosensitive enzymes involved in the mevalonic acid pathway, precursors of terpenes (Gobbo-Neto and Lopes, 2007). Thus, irradiance can influence directly the production of essential oil, or indirectly, through the increase of plant biomass (Pegoraro et al., 2010). Irradiance is essential for plant growth, since it affects the primary metabolism providing energy for photosynthesis and generating signals that regulate their development (Lima et al., 2010).

In 2004, the Agência Nacional de Vigilância Sanitária in Brazil, approved the registration of the first topic antiinflammatory made from the essential oil of the Brazilian plant, *V. curassavica*, whose active ingredient is $\alpha$-humulene. Thus, increasing the need for research on culture and management of this species.

The aim of this study was to evaluate the effect of irradiance on *V. curassavica* growth, trichome frequency, and essential oil yield and composition.

## Materials and methods

### *Plant material*

Seedlings of *Varronia curassavica* Jacq., Boraginaceae, were obtained by seed propagation. A voucher specimen was deposited at the Universidade Estadual de Santa Cruz Herbarium under the accession number 13.895. A month after germination, the plants were transferred to pots containing 10 l of soil and subjected to four treatments of light irradiance (20, 50, 70 and 100% light-full sun) for 90 days. The full sun treatment corresponded to natural irradiance conditions in the Medicinal Plants Garden of Universidade Estadual de Santa Cruz, while the other treatments were obtained using black screens for cover. The photosynthetically active radiation (PAR) in each environment was measured with a quantum sensor BQM-SUN (Apogee, USA) for five days in one-hour intervals from 7 am to 5 pm. The maximum PAR was observed in full sun (2240 $\mu mol.m^{-2}.s^{-1}$) followed by the shaded treatments at 1610, 1120 and 574 $\mu mol.m^{-2}.s^{-1}$, which are 70, 50 and 20% of light, respectively.

At the end of the experiment six plants were harvested to evaluate dry biomass of roots (R), stems (S), leaves (L), total plant (T), leaf area (LA) and leaf mass per area (LMA). The dry biomass was obtained using an oven with air ventilation at 70°C, until constant weight and leaf area was measured, using an electronic leaf area meter, model LI-3100 (Li-Cor, Inc. Lincoln, Nebraska, USA).

### *Isolation and chemical analysis of essential oils*

Leaves of four plants were oven-dried at 40°C with air ventilation for essential oil extraction by hydrodistillation using a Clevenger apparatus. The collected hydrolate was submitted to liquid-liquid partition chromatography with dichloromethane, the organic fractions were separated and dried with anhydrous sodium sulfate, and further concentrated. The mean concentrations of essential oils were calculated as weight of oil (g) per 100 g of dry leaf biomass, and essential oil yield per plant ($g\ plant^{-1}$).

Gas chromatography analyses were performed using a Varian Saturno 3800 gas chromatograph equipped with a flame ionization detector (FID) and a Varian VF-5ms fused silica capillary column (30 m × 0.25 mm; 0.25 µm film thickness). The carrier gas was helium at a flow rate of 1.0 $ml.min^{-1}$. The injector and detector temperatures were 250 and 280°C, respectively. The column oven temperature started at 60°C and increased by 6°C $min^{-1}$ up to 280°C, where it was maintained for 5 min. The sample (1 µl) diluted in 10% ethyl acetate was injected in the split mode (1:10). The concentration of the essential oil constituents was calculated from the peak area in relation to the total area of the sample. The qualitative analysis was performed in a Varian 2000 mass spectrometer, using the 70 eV electronic impact method, scan mass range of 40-450 *m/z* at a sampling rate of 1.2 scan/s, with the transfer line temperatures at 280°C, manifold at 120°C, and trap at 240°C equipped with a VF-rms capillary column (30 m × 0.25 mm × 0.25 µm). The injector temperature was 250°C; the carrier gas, the column temperature and the injection flow were operated under similar GC-FID conditions with an electron impact of 70 eV. Chemical constituents were identified by comparison with the library system (NIST08), literature and Kovats retention index (Adams, 2007).

### *Trichomes distribution*

Fully expanded leaves were collected from the fourth node from the apex to the base of the plant. Segments of the median portion leaf were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 6.9, dehydrated in crescent ethanol series and dried at critical point (CPD 030, Bal-Tec, Balzers, Liechtenstein), and gold coated using a sputter coater apparatus (SCD 050, Bal-tec, Balzers, Liechtenstein). After that, the samples were examined using a scanning electron microscope (JEOL JSM-6390LV). Four replicates were carried out and six observation fields were randomly selected, totalling 24 observation fields per treatment. The trichome frequency was performed in the regions between the leaf veins of both leaf surfaces.

### *Statistical analysis*

All data were subjected to analysis of variance considering the completely randomized design to test the difference between treatments on each variable. Plant growth characteristics, the content and essential oil yield and trichomes frequency were subjected to regression analysis. The mean concentration of the essential constituents oil was compared with Tukey's test (5% significance) using the statistical program SISVAR (Ferreira, 2011).

## Results and discussion

The biomass accumulation of different organs of *Varronia curassavica* Jacq., Boraginaceae, presented different responses to the irradiance levels (Fig. 1). Total dry biomass showed a quadratic fit, with maximum production of 193.96 g at 54% of irradiance. While the stem dry biomass decreased linearly with