

ALLOMETRIC EQUATIONS TO ESTIMATE THE LEAF AREA OF
Psychotria colorata (WILLD. EX SCHULT.) MÜLL.ARG.

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Abstract

Estimating leaf area using non-destructive methods from regression equations has become a more efficient, quick, and accurate way. Thus, this study aimed to propose an equation that significantly estimates the leaf area of *Psychotria colorata* (Rubiaceae) through linear leaf dimensions. For this purpose, 200 leaves of different shapes were collected, and length (L), width (W), product of length by width (L.W), and real leaf area (LA) of each leaf blade were determined. Then, equations were adjusted for predicting leaf area using simple linear, linear (0.0), quadratic, cubic, power, and exponential regression models. The proposed equation was selected according to the coefficient of determination (R^2), Willmott's agreement index (d), Akaike's information criterion (AIC), mean absolute error (MAE), mean squared error (RMSE) and BIAS index. It was noted that the equations adjusted using L.W met the best criteria for estimating leaf area, but the equation $LA = 0.59 * L.W$ from linear regression without intercept was the most suitable. This equation predicts that 59% of leaf area is explained by L.W. Concluding, the leaf area of *P. colorata* can be estimated using an allometric equation that uses linear leaf blade dimensions.

Keywords: Allometric equations. Leaf blade. Modeling. Perpétua-do-mato. Rubiaceae.

1. Introduction

Popularly known as "Perpetua-do-mato" and "repolho", *Psychotria colorata* (Rubiaceae) is an understory shrub, 1.0 m meter height, that owns simple leaves, pink or purple flowers, and elliptical and blue fruits (Taylor et al. 2007; Vicentini et al. 2016). It is a native and non-endemic species widely distributed in South America, occurring in Guyana, Venezuela, and North, Northeast, and Midwest regions of Brazil (Zappi et al. 2015). The plant is of economic importance and used mainly by traditional populations as a medicinal plant. Due to alkaloids in its leaves, flowers, fruits, and roots, the plant has analgesic properties, being used mainly for ear treatments and abdominal pain alleviation (Matsuura et al. 2013; Souza et al. 2013; Moura and Maruo 2014; Skiryecz et al. 2016).

Given the importance of *P. colorata*, ecophysiological studies on growth and propagation of this species become necessary. This way, estimating leaf area is essential to study plant growth and development because leaves directly influence on photosynthetic efficiency, gas exchange, biotic and abiotic stress regulation (Benincasa 2003; Spann and Heerema 2010; Candido et al. 2013).

Leaf area can be determined through destructive (direct) and non-destructive (indirect) methods (Malagi et al. 2010; Sousa et al. 2015). Direct methods are simple and precise, but require more time to be

performed, in addition to lead to plant destruction (Leite et al. 2017). On the other hand, indirect methods have become as efficient as direct methods, in addition to becoming more practical and accurate, without destroying the sample and allowing many measurements on the same plant at a given time (Pompelli et al. 2012; Sarker et al. 2013). Thus, regression equations have been used to estimate leaf area using linear dimensions of leaves, that is limb length and width (Zhang and Pan 2011).

The non-destructive method was used to estimate leaf area in many medicinal plants, such as *Crotalaria juncea* L. (Carvalho et al. 2017a), *Rosa hybrida* L. (Gao et al. 2012), *Calotropis procera* (Aiton) W. T. Aiton. (Ferreira et al. 2015), *Solanum melongena* L. (Hinnah et al. 2014), *Brassica napus* L. (Tartaglia et al. 2016), *Plectranthus ornatus* Codd. (Silva et al. 2017), *Commelina difusa* Burm.f. (Carvalho et al. 2017b), *Erythroxylum citrifolium* A.St.-Hil. (Ribeiro et al. 2019a) and *Erythroxylum simonis* Plowman (Ribeiro et al. 2018a). Similarly, this study aimed to obtain a regression equation that meaningfully estimates leaf area of *Psychotria colorata* from linear dimensions of leaf blades.

2. Material and Methods

The research was conducted at the Mata do Pau-Ferro Study Park, (6°58'12"S, 35°42'15"W), in the municipality of Areia, Paraíba state, Northeast region, Brazil (Figure 1). The municipality is situated in the microregion of Brejo Paraibano, Agreste mesoregion, reaching between 400 and 600 m in altitude, average air temperature of 22 °C and average annual precipitation of approximately 1,400 mm (Ribeiro et al. 2018b). The region's climate is classified as Aw', according to Peel et al. (2007), being tropical hot and humid with autumn-winter rains.

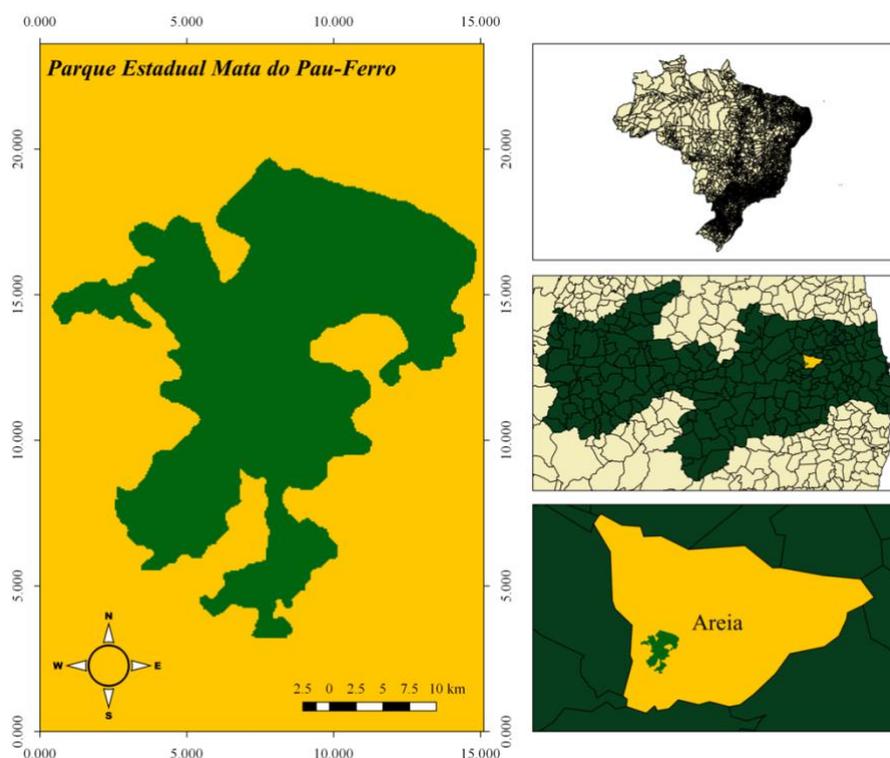


Figure 1. Location of Parque Estadual Mata do Pau-Ferro, Areia, Paraíba, Brazil.
Source: João Everthon da Silva Ribeiro (2020).

For measurement, 200 leaves of different physiognomies were collected in 25 plants from *P. colorata*, selecting those healthy limbs, without biotic and abiotic injuries (Schmidt et al. 2014). The leaves were packed in plastic bags inside ice-filled containers to prevent excessive water loss, then transported to the Laboratory of Vegetal Ecology, at the Federal University of Paraíba, for further analysis. At the laboratory, length (L) and width (W) of each leaf blade were measured using a digital caliper, as shown in Figure 2, and the product of these two variables was then calculated (L.W). Also, to obtain the real leaf area (LA), the 200 leaves were photocopied at 300 dpi resolution using a scanner, and the images were processed using the *ImageJ*® program (Collins 2007), a public domain software.

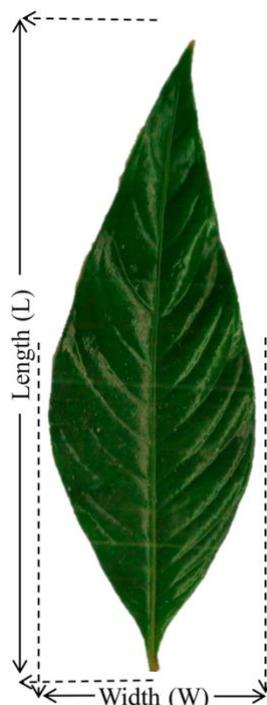


Figure 2. Length and width of leaf blades used to estimate leaf area of *P. colorata*.

A preliminary descriptive analysis for length (L), width (W), product of length by width (L.W), and leaf area (LA) from the 200 leaf blades was performed, wherewith minimum value, maximum value, mean, median, total amplitude, variance, standard error, standard deviation, and coefficient of variation for each variable were calculated.

To find an equation that accurately estimates the leaf area of *P. colorata*, linear, linear without intercept (0.0), quadratic, cubic, power, and exponential regressions models were fitted using the linear dimensions of the leaf blades, as described in Table 1. Afterward, the regression equation that best estimate the leaf area was selected according to highest determination coefficient (R^2) and highest Willmott index (d) (equation 1) (Willmott et al. 1981), lowest mean absolute error (MAE) (equation 2), lowest Akaike information criterion (AIC) (equation 3) (Akaike 1974), and lowest root mean square error (RMSE) (equation 4) (Janssen and Heuberger 1995); and BIAS index closest to zero (equation 5) (Leite and Lima 2002). Statistical analyses were performed in R° software v.3.5.1.

$$d = 1 - \frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{\sum_{i=1}^n (|\hat{y}'_i| + |y'_i|)^2} \quad (1)$$

$$MAE = \frac{\sum_{i=1}^n |\hat{y}_i - y_i|}{n} \quad (2)$$

$$AIC = -2 \ln L(x \setminus \hat{\theta}) + 2(p) \quad (3)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}} \quad (4)$$

$$BIAS = \frac{\sum_{i=1}^n (\hat{y}_i - y_i)}{\sum_{i=1}^n (y_i)} \quad (5)$$

\hat{y}_i : estimated leaf area values; y_i : observed leaf area values; \bar{y}_i : mean of observed values; $\hat{y}'_i = \hat{y}_i - \bar{y}$; $y'_i = y_i - \bar{y}$; $L(x \setminus \hat{\theta})$: maximum likelihood function, defined as the product of density function; p : n° of model parameters; n : total number of observations.

Table 1. Regressions models used to estimate the leaf area (LA) of *P. colorata* using linear dimensions (L, W and L.W) of its leaf blades.

Model	Equation
Linear	$LA = a + b*L$
Linear	$LA = a + b*W$
Linear	$LA = a + b*(L.W)$
Linear without intercept (0.0)	$LA = b*(L.W)$
Quadratic	$LA = a*L^2 + b*L + L$
Quadratic	$LA = a*W^2 + b*W + L$
Quadratic	$LA = a*(L.W)^2 + b*(L.W) + L$
Cubic	$LA = a*L^3 + b*L^2 + L*L + d$
Cubic	$LA = a*W^3 + b*W^2 + L*W + d$
Cubic	$LA = a*(L.W)^3 + b*(L.W)^2 + L*(L.W) + d$
Power	$LA = a*L^b$
Power	$LA = a*W^b$
Power	$LA = a*(L.W)^b$
Exponential	$LA = a*b^L$
Exponential	$LA = a*b^W$
Exponential	$LA = a*b^{(L.W)}$

3. Results and Discussion

Length of limbs ranged from 2.74 to 25.16 cm, 10.97 cm mean and 22.41 cm amplitude, while width varied from 0.63 to 6.90 cm, 3.15 cm on mean and 6.26 amplitude. Product of length by width varied from 1.81 to 173.77 cm², 41.14 cm² on mean and 171.96 cm² amplitude; and real leaf area ranged from 1.37 to 102.64 cm², 24.65 cm² on mean and 101.27 cm² amplitude (Table 2).

The lowest coefficients of variation were found for L (43.38%) and W (46.56%), whereas the highest coefficients were those from L.W (82.49%) and LA (81.75%) (Table 2). High values of standard error, total amplitude, standard deviation, and coefficient of variation are fundamental for estimating leaf area through regression models (Levine et al. 2017).

High variation in data meets more representative regression models, thus allowing researchers to measure the plant at different growth stages (Pezzini et al. 2018). Therefore, the number of leaves (200) evaluated in the present study was meaningful for estimating the leaf area of *P. colorata* using dimensions of its leaf blades. Moreover, high variation in product of length by width and real leaf area was also observed in previous studies (Leite et al. 2017; Ribeiro et al. 2018a; Ribeiro et al. 2020a).

Table 2. Minimum, maximum, mean, total amplitude, median, variance, standard deviation, standard error, and coefficient of variation for length (L), width (W), product of length by width (L.W), and real leaf area (LA) of 200 *Psychotria colorata* leaf blades.

Descriptive statistics	L	W	L.W	LA
Minimum	2.748	0.636	1.8137	1.375
Maximum	25.167	6.905	173.7781	102.649
Mean	10.972	3.1527	41.1459	24.6517
Median	10.2465	2.9985	30.7256	18.682
Total Amplitude	22.419	6.269	171.9645	101.274
Variance	22.6566	2.155	1152.096	406.1181
Standard Deviation	4.7599	1.468	33.9425	20.1524
Standard Error	0.3366	0.1038	2.4001	1.425
Coefficient of Variation	43.38%	46.56%	82.49%	81.75%

Scatterplots between L, W, L.W, and LA showed different dispersion patterns (Figure 3), indicating that the data fit linear and non-linear regression models, as found in other similar studies (Toebe et al. 2019; Ribeiro et al. 2020b).

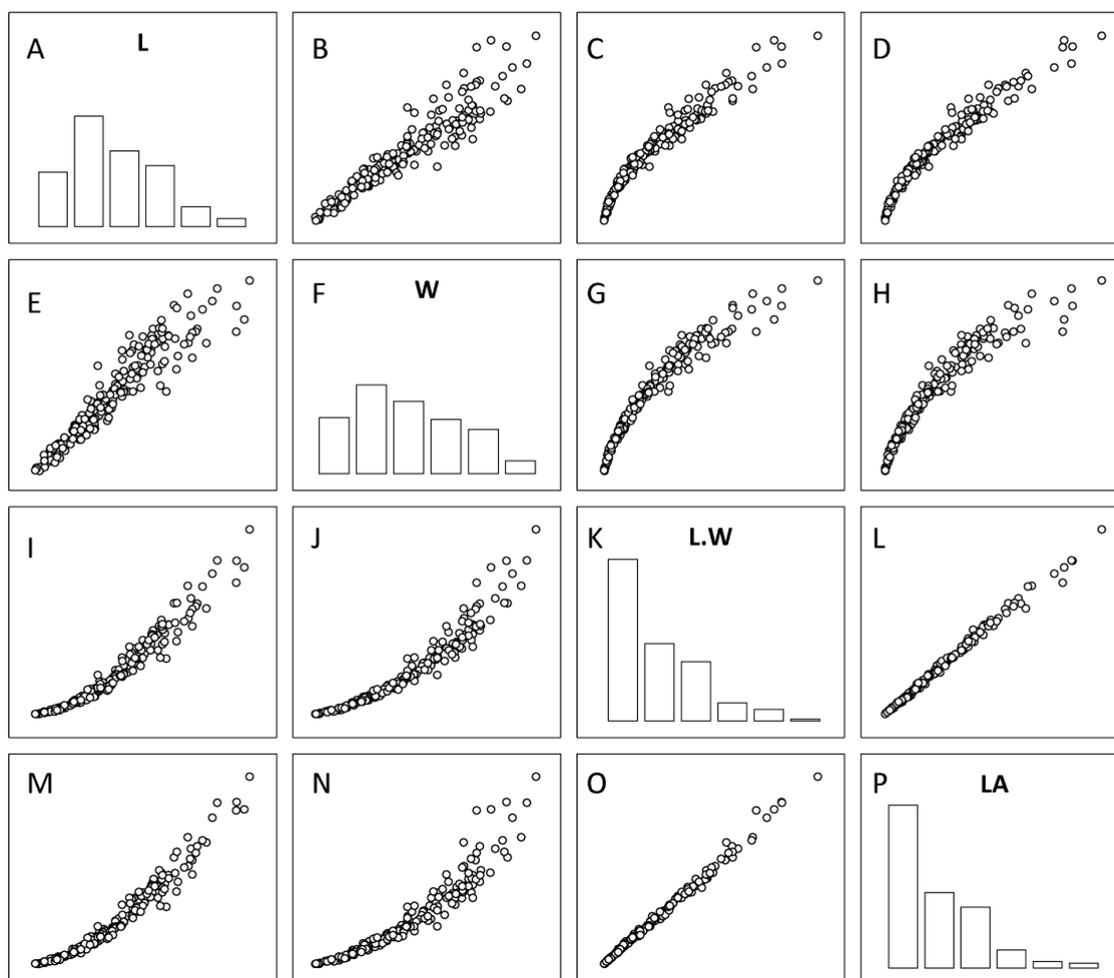


Figure 3. Frequency histogram and scatterplots between length (L), width (W), product of length by width (L.W), and real leaf area (LA) of 200 *Psychotria colorata* leaf blades. Frequency histogram: A – length; F – width; K – product of length by width; P – real leaf area. Scatterplots, relationship between: B, E – L and W; C, I – L and L.W; D, M – L and LA; G, J – W and L.W; H, N – W and LA; L, O – L.W and LA.

Figure 4 shows the leaf area percentage distribution by different intervals. It was noted that 25% of the real leaf area was between 10.01 and 20.00 cm², suggesting that *P. colorata* leaves are medium in size. Ribeiro et al. (2019b), observed that *Psychotria carthagenensis* Jacq. and *Psychotria hoffmannseggiana* (Willd. ex Schult.) Müll.Arg., other species from the Rubiaceae family, own leaves of different sizes.

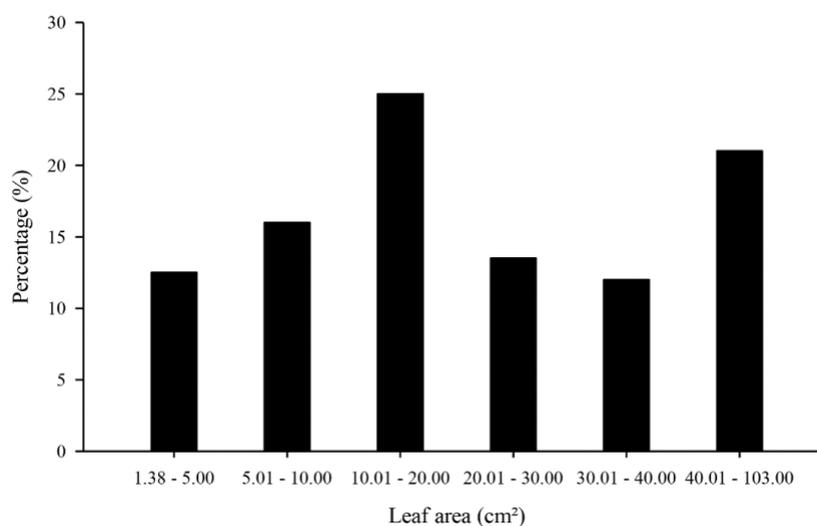


Figure 4. Leaf area percentage distribution of 200 *Psychotria colorata* leaf blades by different intervals.

Table 4 shows the equations adjusted using regression models for predicting leaf area using linear dimensions of leaves. All proposed equations meaningfully estimated *P. colorata* leaf area, with high determination coefficients ($R^2 > 0.88$). Thus, at least 88% variation in leaf areas were explained by the proposed allometrics equations. The equations using L.W met the best assumptions for predicting leaf area than those that used L and W, better fitting the regression models (Mota et al. 2014; Assis et al. 2015; Braga et al. 2018; Lucena et al. 2018; Pezzini et al. 2018; Leite et al. 2019; Oliveira et al. 2019a; Ribeiro et al. 2019a). Though, except for the exponential model, that was best fitted using L.

Therefore, among the regressions models studied, the linear model without intercept using L.W showed the highest values determination coefficient ($R^2 = 0.9978$), highest Willmott index ($d = 0.9987$), lowest Akaike information criterion (AIC = 724.12), lowest root mean square error (RMSE = 1.4494), lowest mean absolute error (MAE = 0.8884), and BIAS index closest to zero (0.1084) (Table 4). This way, the equation $LA = 0.59 * L.W$ was that best estimated *P. colorata* leaf area, becoming a quick and practical way to do so. Also, this equation predicts that 59% of the leaf area can be explained by the product between length and width. This same regression model was used to estimate leaf area of other medicinal plants, such as *Capsicum annum* L. ($LA = 0.57 * L.W$; Padrón et al. 2016), *Combretum leprosum* Mart. ($LA = 0.7103 * L.W$; Candido et al. 2013), *Commelina diffusa* Burm.f. ($LA = 0.7 * L.W$; Carvalho et al. 2017b), *Crotalaria juncea* L. ($LA = 0.7390 * L.W$; Carvalho et al. 2017a), *Ceiba glaziovii* (Kuntze) K. Schum. ($LA = 0.4549 * LW$; Ribeiro et al. 2020c), and *Erythroxylum simonis* Plowman ($LA = 0.6426 * LW$; Ribeiro et al. 2018a).

Table 4. Regression models, linear dimensions (x), determination coefficient (R^2), Akaike information criterion (AIC), root mean square error (RMSE), mean absolute error (MAE), Willmott index (d) and BIAS index from linear dimensions of 200 *Psychotria colorata* leaf blades.

Model	x	R^2	AIC	RMSE	MAE	d	BIAS	Equation
Linear	L	0.9380	1216.90	4.9943	3.8152	0.9838	0.4396	$LA = -20.34 + 4.10 * L$
Linear	W	0.8888	1333.68	6.6876	4.6069	0.9698	0.4986	$LA = -16.16 + 12.95 * W$
Linear	L.W	0.9945	733.58	1.4993	0.9406	0.9986	0.3678	$LA = 0.29 + 0.59 * LW$
Linear (0.0)	L.W	0.9978	724.12	1.4494	0.8884	0.9987	0.1084	$LA = 0.59 * LW$
Quadratic	L	0.9673	1089.59	3.6148	2.4738	0.9917	0.3101	$LA = -5.05 + 1.14 * L + 0.12 * L^2$
Quadratic	W	0.9323	1235.30	5.2033	3.0303	0.9823	0.3002	$LA = 1.15 + 0.31 * W + 1.86 * W^2$
Quadratic	L.W	0.9947	732.55	1.4880	0.8944	0.9986	0.1642	$LA = 0.77 + 0.56 * LW - 0.0002 * LW^2$
Cubic	L	0.9689	1082.37	3.5324	2.2890	0.9921	0.3597	$LA = 1.28 - 0.79 * L + 0.28 * L^2 - 0.004 * L^3$
Cubic	W	0.9328	1234.83	5.1713	2.9470	0.9826	0.3125	$LA = -2.86 + 5.04 * W + 0.32 * W^2 + 0.14 * W^3$
Cubic	L.W	0.9947	726.04	1.4497	0.8885	0.9986	0.1169	$LA = 0.72 + 0.57 * LW + 0.0001 * LW^2 - 0.0000004 * LW^3$
Power	L	0.9670	1093.87	3.6720	2.5129	0.9913	-0.2454	$LA = 0.26 * L^{1.85}$
Power	W	0.9325	1235.42	5.2310	3.0875	0.9823	0.4200	$LA = 2.39 * W^{1.88}$
Power	L.W	0.9945	735.56	1.4992	0.9371	0.9986	0.1736	$LA = 0.60 * LW^{1.00}$
Exponential	L	0.9181	1289.91	5.9944	4.8632	0.9745	-0.9643	$LA = 5.81 * 1.13^L$
Exponential	W	0.9164	1284.03	5.9070	3.9625	0.9759	-0.6288	$LA = 4.92 * 1.56^W$
Exponential	L.W	0.9165	1361.93	5.9070	3.9625	0.9759	-0.6288	$LA = 12.76 * 1.01^{LW}$

Low data dispersion from the regression line indicates that the equation $LA = 0.59 * L.W$ can significantly estimate *P. colorata* leaf area (Figure 5). Also, the leaf area estimated by this equation significantly equaled the real leaf area, with 0.9945 determination coefficient (R^2) (Figure 6). This proposed regression equation might contribute to environmental studies that focus on the growth, development, and propagation of *P. colorata*, assisting in the conservation of the species in forest remnants.

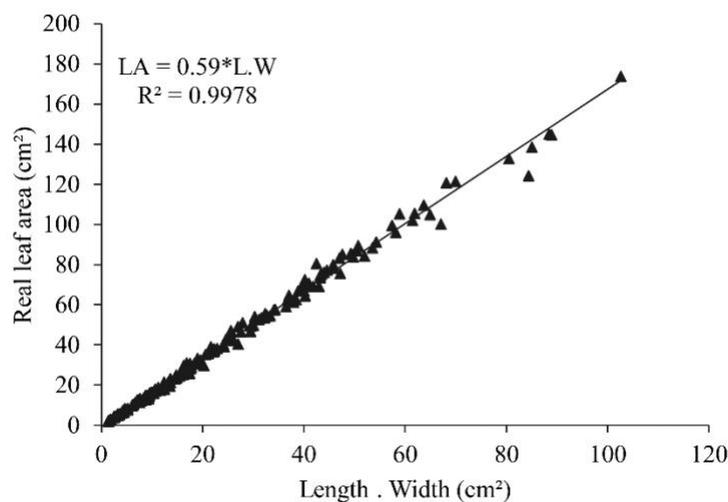


Figure 5. Real leaf area (LA) of *Psychotria colorata* as a function of the product of length by width (L.W) of leaf blades, using the proposed equation $LA = 0.59 * L.W$ for estimating leaf area.

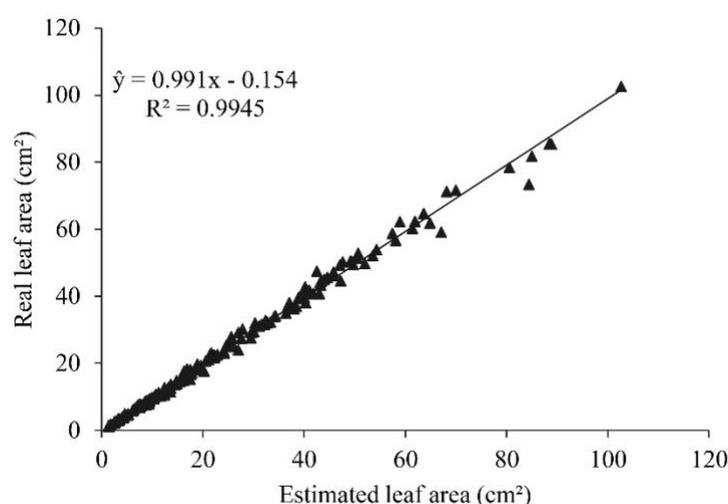


Figure 6. Relationship between real leaf area of *Psychotria colorata* and estimated leaf area using the proposed equation $LA = 0.59 * L.W$.

4. Conclusions

Leaf area of *P. colorata* can be determined by allometric equations, using linear dimensions of the leaf blades.

The equation $LA = 0.59 * L.W$, from the linear regression model without intercept, was that most suitable for estimating *P. colorata* leaf area, as a quick and accurate way.

Regression equations that used the product of length by width (L.W) better estimated the leaf area of the species.

Authors' Contributions: RIBEIRO, J.E.S.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, critical review of important intellectual content; FIGUEIREDO, E.S.: acquisition of data, drafting the article, and critical review of important intellectual content; COELHO, E.S.: drafting the article, and critical review of important intellectual content; MELO, M.F.: critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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