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A linear model for leaf area measurement to screen potential leaf material for herbal drug in *Adhatoda vasica* L.

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Abstract: Leaf area is an important parameter in physiology and agronomy studies. Linear models for leaf area measurement are developed for plant species as a nondestructive method. The plant *Adhatoda vasica* L. (a medicinal plant) was selected and the leaves of this plant were used for development of linear model for leaf area using Leaf Area Meter (LAM) software. Planimetric parameters (length, length^2, width and width^2) and gravimetric (dry weight and water content) parameters are considered for the development of linear model for this plant species. Single factor ANOVA and linear correlations were worked out using these parameters and leaf area. The plant was showed significant relationship with the parameters studied. The best correlation as represented by regression coefficient (R^2) was used and improved R^2 is worked out. It is observed that with increase in leaf area, water content is also increased and showed best correlation with the leaf area. Thus water content can be taken as a parameter for developing linear model for leaf area is concluded.

Keywords: *Adhatoda vasica* L., Gravimetric parameters, Leaf area, Planimetric parameters, Regression coefficient

INTRODUCTION

Adhatoda vasica belonging to family Acanthaceae, commonly known as Adosa, is a small, evergreen shrub found many regions of India and throughout the world, with a multitude of uses in traditional Ayurveda (Gangwar and Ghosh, 2014). Leaves are large and lance-shaped. Stem is herbaceous above and woody below. Leaves opposite and exstipulate. Flower spikes or panicles, small irregular zygomorphic, bisexual, and hypogynous (Shinwari and Shah, 1995). The leaves, flowers, fruit and roots are extensively used for treating cold cough, whooping cough, chronic bronchitis and asthma, as sedative, expectorant and antispasmodic (Pandita *et al.,* 1983). The vast variety of pharmacological uses of *Adhatoda* is believed to be the result of its rich concentration of alkaloids (Shrivastava *et al.,* 2006; Maikhuri and Gangwar, 1965). *Adhatoda vasica* used for its anti-asthmatic and bronchodilator activity (Lahiri and Pradhan, 1964), wound healing activity (Bhargava *et al.,* 1988), anti-ulcer activity (Shrivastava *et al.,* 2006), anti allergy activity (Wagner, 1989), anti bacterial activity (Patel and Venkatakrishna, 1984), cholagogue activity (Rabinovich *et al.,* 1966), antitubercular activity (Narimaian *et al.,* 2005), insecticidal activity (Srivastava *et al.,* 2006) and abortifacient and uterotonic activity (Claeson *et al.,* 2000). This plant has great potential to be developed as drug pharmaceutical industries. However, herbal formulations are often questioned for its consistency mainly because of less care is taken in antidote preparations. Physiologically active compounds may vary with age and conditions of the leaves; therefore, there must be some guide lines for the collection of the materials. Leaf area of the plants not only determines the photosynthetic capacity but can be use as a morphological marker for collection of medicinally important materials (Kuvad *et al.,* 2014; Tatmiya *et al.,* 2014). Leaf area of the plants is correlated with leaf dimensions like length and width and linear models are developed as non-destructive method. Further, gravimetric parameters like leaf dry weight and water content can also be used to build linear model for leaf area (Daughtry, 1990). Leaf area (LA) is an important variable for most ecophysiological studies in terrestrial ecosystems concerning light interception, evapotranspiration, photosynthetic efficiency, fertilizers, irrigation response and plant growth (Blanco and Folegatti, 2003) and hence also to maintain herbal drug farms with efficient practices. Therefore, in this study, a linear model for leaf area measurement is developed for *Adhtoda vasica* plant which can be useful for leaf harvest for the best pharmaceutical preparations.

MATERIALS AND METHODS

Collection of the samples: The plant material (leaves) of *A. vasica* was collected from the botanical garden of Department of Biosciences, Saurashtra University,

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Table 1. Regression analysis between leaf area and gravimetric/planimetric parameter of *Adhatoda vasica* L.

Parameter	Y	R^2	R	OBSàLAC R^2	R
Gravimetric parameter					
DW	y = 11701x + 1223	R^2 = 0.824	0.907	R^2 = 0.841	0.917
WC	y = 5666.x + 534.5	R^2 = 0.908	0.952	R^2 = 0.979	0.989
Planimetric parameter					
L	y = 144.5x – 3133	R^2 = 0.826	0.908	R^2 = 0.806	0.897
L^2	y = 1.371x + 458.6	R^2 = 0.862	0.928	R^2 = 0.985	0.992
W	y = 444.0x – 2954	R^2 = 0.803	0.896	R^2 = 0.819	0.904
W^2	y = 12.61x + 711.9	R^2 = 0.801	0.984	R^2 = 0.958	0.979

Note: n=Number of leaf (130); DW= Dry Weight; WC= Water Content; L= Length; L^2= Length Square; W= Width; W^2= Width Square; OBS= Observed value; LAC= Leaf Area Calculated.

Rajkot (22.30°N, 70.78°E), Gujarat, India. Nearly 130 leaves were taken for this study.

Measurement of leaf area: Collected leaves are washed thoroughly under running tap water and blotted over filter papers. Each individual leaf/leaflets were scanned and saved as .bmp file. The scanned leaf is weighed before and after oven drying at 80 °C for constant weight. The difference in fresh and dry weight is considered as water content. Maximum width (W) and length of mid rib (L) was measured. All data tabulated for L, L^2, W, W^2, water content (WC) and dry weight (DW). Each individual leaf/leaflets was scanned and saved as .bmp files then proceed for leaf area measurement as per the software standard protocol (Kuvad *et al*., 2014; Tatmiya *et al*., 2014). The present method of leaf area measurement is very simple, rapid as software (LAM) is very efficient and user friendly (http://btm.gujarat.gov.in/btm/bitvirtual-init.htm). This software measures different parameter of leaf (leaf area, length of midrib and width of leaf) similar measurements can be done using image J software (O'neal *et al*., 2002).

Statistical Analysis: All collected data were subjected to correlation analysis like single factor ANOVA and linear correlations between leaf area and planimetric or gravimetric parameters using Excel software (MS office, Microsoft).

RESULTS AND DISCUSSION

In this study, Gravimetric parameters (WC and DW) and Planimetric parameters (L, L^2, W and W^2) are worked out for correlation analysis showed significant variations (Table 1). All R^2 were statistically significant, therefore the improved R^2 were taken into consideration. Although all regression equations for improved values were calculated, linear model was given for the better relationship on the basis of R^2 obtained. Regression analysis of *A. vasica* plant with gravimetric and planimetric parameters is presented in table-1 (Figs. 1 -3). Data on leaf area and DW showed statistically significant relationship R^2= 0.824 which improved to R^2= 0.841 (Figs. 1A and B). Data on WC showed R^2= 0.908 which improved to R^2= 0.979 when regression equation of first set of data is used to test

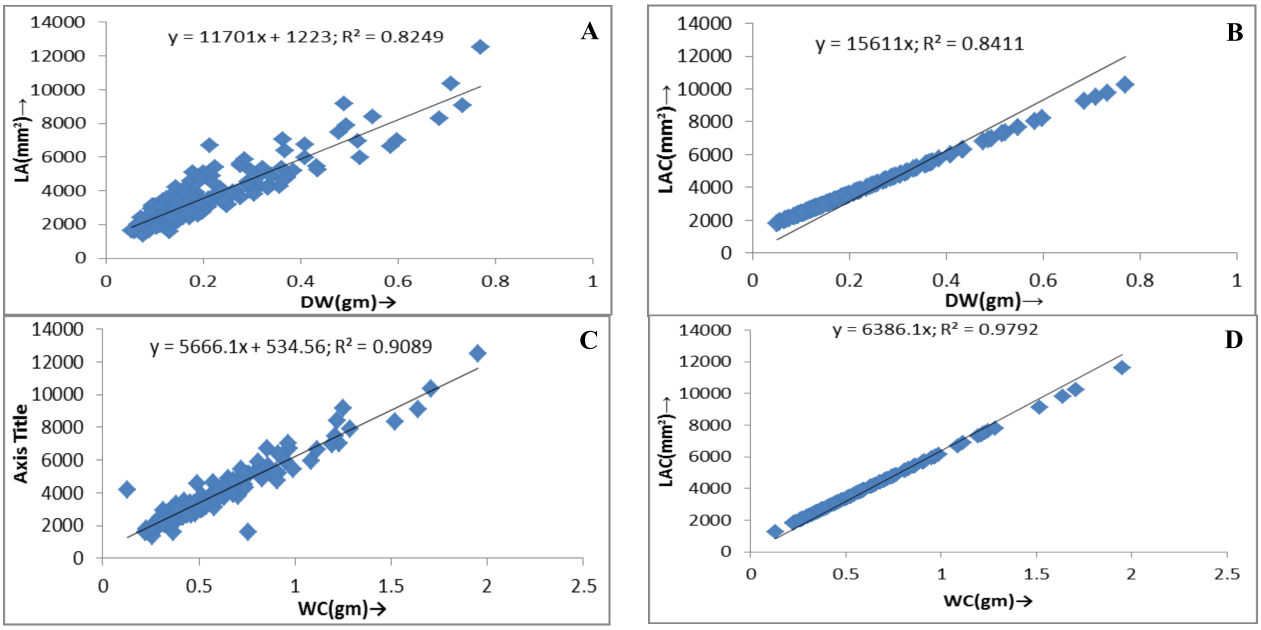

Fig.1. *(A) Leaf area versus dry weight. (B) Calculated value of leaf area versus observed value of dry weight. (C) Leaf area versus water content. (D) Calculated value of leaf area versus observed value of water content.*

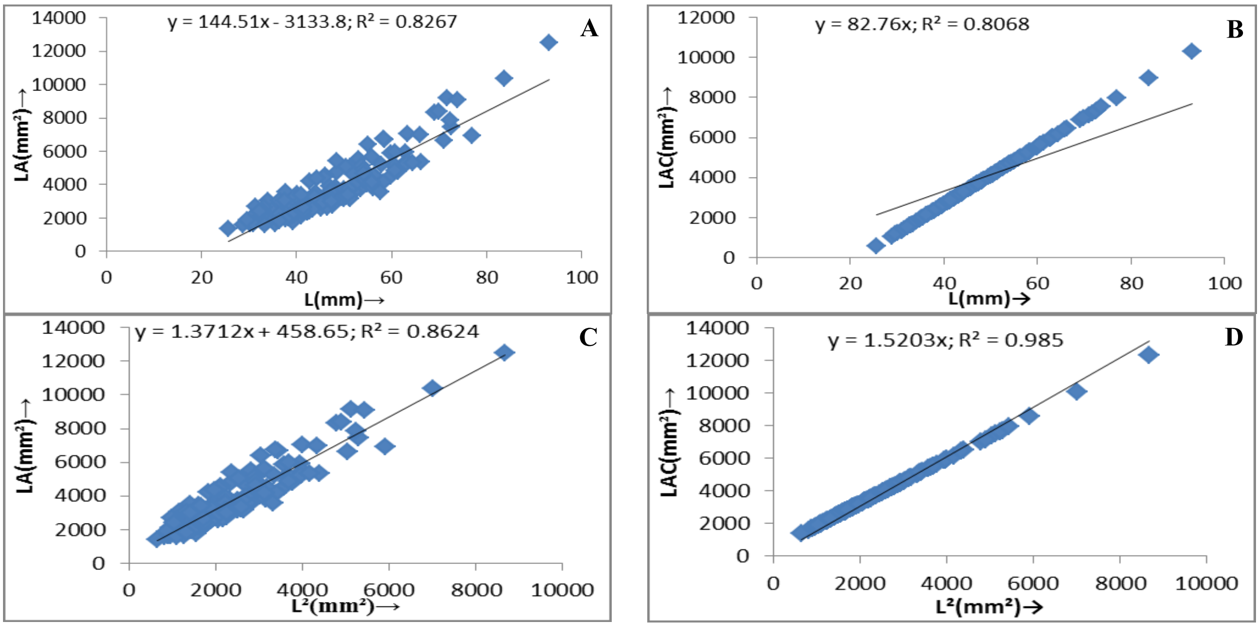

Fig.2. *(A) Leaf area versus midrib length. (B) Calculated value of leaf area versus observed value of midrib length. (C) Leaf area versus midrib length square. (D) Calculated value of leaf area versus observed value of midrib length square.*

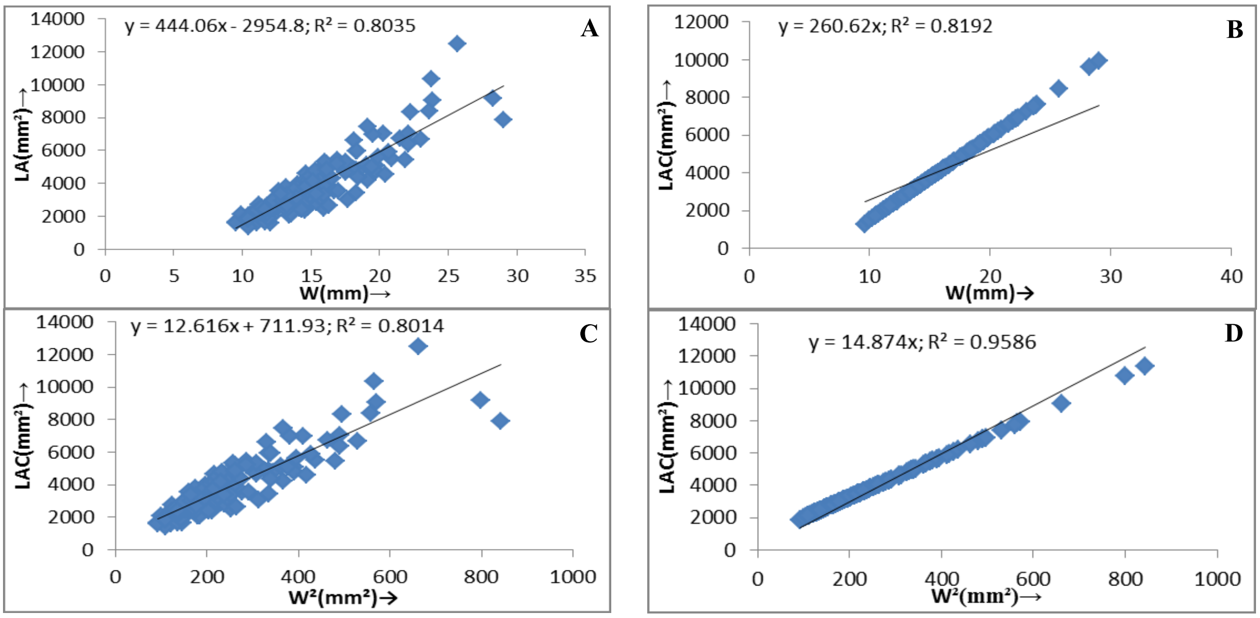

Fig.3. *(A) Leaf area versus leaf width. (B) Calculated value of leaf area versus observed value of leaf width. (C) Leaf area versus leaf width square. (D) Calculated value of leaf area versus observed value of leaf width square.*

the linear model (Figs. 1 C and D).

In gravimetric parameter WC found to be better correlated with leaf area than DW (Fig. 1). When planimetric parameter L and L^2 were correlated with LA, L^2 was better correlated (Figure 2). Regression equation y = 144.5x - 3133 (R^2 = 0.826) for L showed no change relationship or less value for R^2 = 0.806. Data on L^2 showed R^2 = 0.862 which improved to R^2 = 0.985 (n = 130). Similarly data on W correlated with LA plotted in Figure 3 showed R^2 = 0.803 which improved to R^2 = 0.819. Data on W^2 showed R^2 = 0.801 which improved to R^2= 0.958. Thus data on L^2 are better correlated in planimetric parameter.

Water content is also considered as one of the important parameters to study since in many experiments performed in our lab or elsewhere suggests importance of water in expansion growth (Egli and Tekrony, 1997; Rabadia *et al.*, 1999). It is assumed that increase in the size of the leaf may also increase in water content at least up to certain level, the level where leaf size stabilized. Indeed, when gravimetric parameters are considered, we found good correlation with water amount and leaf area in *A. vasica* (Figs. 1C and D, Table 1). Similarly, in planimetric parameters; it is best explained by L^2 in *A. vasica* (Figs. 2C and D, Table 1). The R^2 of planimetric parameter was less as compared to gravimetric. Linear model of these parameters were tested with the leaves of another field which showed improved relationship. Water content is known to play an important role in cell expansion in numbers of the tissues (Egli and Tekrony, 1997; Jhala and Thaker, 2015); it also showed relationship with leaf area in the present study (R^2 = 0.908) and the relationship observed more as compared to dry weight (R^2 = 0.824). In many studies, were gravimetric parameters are taken for consideration; dry weight is reported as a considerable measured (Dingkuh., 1996, Folkard *et al.,* 1999; Wilson *et al*., 2001; Young *et al.,* 2007). However, for *A. vasica* water content was closely related with leaf area than dry weight. Since amount of the water is highly correlated with size of the organs in the study reported above, and in our results suggests that the water content can be taken as a one of the parameter for determination of leaf area by linear model. Additionally our study reported that the water content is statistically more significant than dry weight.

Conclusion

It is suggested that water content per leaf can be considered for development of linear model in *A. vasica* plant since it is more statistically significant than any other parameter in *A. vasica* plant. The results on leaf area can be used as guidelines for the harvest of *A. vasica* plant for effective pharmaceutical preparations.

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