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**PLANT PHYSIOLOGY**

# Standardization of Linear Model for Leaf Area Measurement for Medicinally Important Plants: *Balanites aegyptiaca* L. and *Gymnosporia montana* (Roth.)

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## ABSTRACT

Leaf area measurement is a key parameter for physiological, agronomical, and ecological studies. India has covered the 38 percent geographical area of the arid and semi-arid region. This region is very rich in plants biodiversity. In this work the medicinal plants of semi-arid region was selected for the linear model development for the leaf area. In the present study different sizes of healthy leaves of *Balanites aegyptiaca* and *Gymnosporia montana* were collected and the fresh and dry weights, water content, chlorophyll content, and leaf area were measured. Further, the dry weight and water content were considered for the development of the linear model for these plant species which showed a highly statistically significant relationship with leaf area. It was observed that fresh and dry weights, water content gradually increased with leaf size. The best correlation was represented by regression coefficient $R^2$ and correlation coefficient *r*. The results concluded that dry and fresh weights and water content either one, can be selected as a parameter for developing the linear model for leaf area in both these plants. The probable role of these parameters with leaf size and their application in the herbal formulation is discussed.

## HIGHLIGHTS

- Leaf area linear model developed for medicinal plant *B. aegyptiaca* & *G.montana.*
- Significant relationship was observerd leaf area with dry weight and water content.

**Keywords**: Gravimetric parameters, medicinal plants, leaf area, linear model, the regression coefficient

Plants are one of the most important sources of medicines. Medicinal plants are used for thousands of years in cure of many diseases. Ayurveda, Siddha and Unani, the oldest medical system in India, has reported more than 20,000 medicinal plant species (Pandey *et al.* 2013). India can play the lead role in the production of standardized, therapeutically effective preparation of herbal formulations in the world. For the preparation of effective herbal formulation, a proper selection of plant materials is needed.

Leaf area is an important parameter for studies of physiological processes including the growth, photosynthesis, respiration and transpiration, plant nutrition, light interception, energy balance, biomass accumulation and plays a key role in understanding crop growth and its environment (Kumar 2009; Bakhshandeh *et al.* 2011) and hence also to maintain herbal farms with efficient practices. The leaf area of a plant is correlated with width, length, branch length, and number, and plant height and linear

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models are developed as non-destructive methods (Peksen 2007; Akram-Ghaderi and Soltani 2007). Further different parameters like leaf dry weight, water content, or total above-ground biomass dry matter can also be used to build a linear model for leaf area (Bhatt and Chanda 2003; Mokhtarpour *et al.* 2010). These plants have vast potential to be developed as a drug in the pharmaceutical industry. The leaf area of the plants not only determines the photosynthetic capacity but it can also be used as a morphological marker for the selection of proper medicinally important plant materials for effective formulation (Kuvad *et al.* 2014; Tatmiya *et al.* 2014). Each leaf of the plant does not contain the same concentration of physiologically active compounds, but may vary with the size. In the present study, two plants, *Balanites aegyptiaca* L. and *Gymnosporia montana* (Roth.) were selected for the experiment. The leaves of both the plants have very high medicinal value.

*B. aegyptiaca* (desert date), is a tree, which belongs to the family Zygophyllaceae. All parts of plants i.e., root, stem bark, leaves, and fruit pulppossess medicinal properties. *B. aegyptiaca* is containing steroidal, vitamin-C, carbohydrates, saponins, and organic acids (Nour and Salah 2017). The most important components of balanites are saponins, it is composed of sugar and chemically linked with steroid aglycones. This chemical is used in the pharmaceutical industry for the manufacture of steroid drugs (Chen and Wu, 1994; Dangi *et al.* 2014). This plant has been used to cure sickle cell disease, skin boils, malaria, syphilis, spleen disorders, yellow fever, and jaundice and is also used for the treatment of AIDS and Leukaemia type of disease (Yadav and Panghal, 2010; Singh *et al.* 2017). *G. montana* commonly known as Vikalo is an herbaceous plant of Celastraceae. The leaf, flower, and fruits possess antibacterial and antioxidant activity (Dhuru *et al.* 2012). This plant is a good source of flavonoids and is used for the treatment of jaundice, inflammation, and various disorders (Pullaih 2006; Dhru *et al.* 2011).

Considering this, to standardized the leaf size for the collection of pharmaceutically useful material,a linear model for leaf area measurement is developed for *B. aegyptiaca* and *G. montana* plants which may be helpful to the researchers for effective herbal formulation.

## MATERIALS AND METHODS

### Collection of Plant materials

From the Botanical Garden of the Saurashtra University campus, randomly 200 leaves of each plant i.e. *Balanites aegyptiaca* L. and *Gymnosporia montana* (Roth.) were collected. It was wash though roughly under tap water and blotted dry.

### Identification and confirmation of the sample

The plant was authenticated by Prof. Vrinda Thaker, Department of Biosciences, Saurashtra University and a Voucher specimen (CPBGE – 201 and 202) are deposited in the herbarium of the same Department.

### Growth analysis

Growth was measured in terms of fresh and dry weights and water content. Freshly separated leaves were weighed before and after oven drying to a constant weight at 60 °C for 72 hours to obtain the data on dry weights. The water content of each stage was determined by the difference in fresh and dry weights. Data were taken in triplicate and calculated with ± standard deviations.

### Leaf area measurement

Freshly collected 200 leaves were washed thoroughly under running tap water and blotted over filter papers. Each individual leaf was scanned according to the size and saved as .bmp file format. Leaf area was measured by Leaf area meter software Muchadia and Thaker (2017).

### Determination of Chlorophyll content

For the measurement of chlorophyll content, the frequency distribution of leaf on the basis of their size was done and distributed in to separate 5 groups (*B. aegyptiaca* L) and 4 groups (*G. montana*) (Plate 1). The chlorophyll content was determined according to Monpara *et al.* (2018) using the spectrophotometric method. The two wavelengths of absorbance at 645 and 663 nm were recorded and chlorophyll content per mg fresh weight was calculated for each leaf group in three replicates for both the plants.

## STATISTICAL ANALYSIS

A correlation was worked on outgrowth parameters

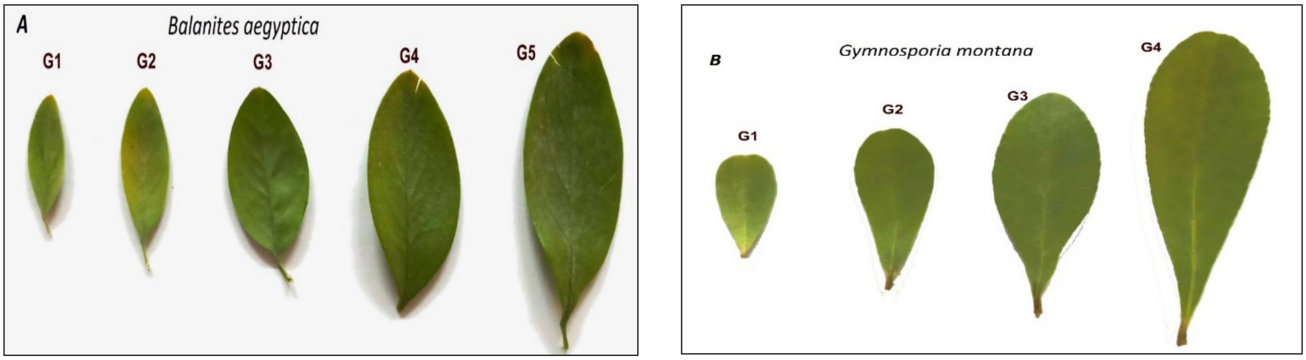


**Plate 1:** Different size of leaves for chlorophyll estimation **(A)** *B. aegyptiaca* **(B)** *G. montana*

i.e. fresh weight, dry weight, water content, and chlorophyll with leaf area using the Excel 2007. P values significant at 0.1 or less were considered for the data interpretation.

## RESULTS AND DISCUSSION

Medicinal plants constitute an effective source of traditional and modern medicine. Leaves of plants have been used as source of medicine. Plant leaves have ability to synthesize a wide variety of biologically important functional compounds. This desired compounds may not have required concentration throughout the growth and development period of the leaf. Age of the plants, which is selected for producing herbal formulations, is needed to be considered for the higher yield of the desired active compounds, which may influence the value of the medicine (Kuvad *et al.* 2014; Tatmiya *et al.* 2014). In the present study, healthy leaves of *B. aegyptiaca*, and *G. montana* were collected for the analysis of growth parameters and chlorophyll content. Plant growth was measured as fresh and dry weight and water content (mg/leaf) and leaf area ($cm^2$). These plants are used for the treatment of various diseases in different part of the world (Dhru *et al.* 2011; Dangi *et al.* 2014).

In this work, randomly selected 200 small to large sized leaves of *B. aegyptiaca* were used for the leaf area measurements by using Leaf Area Measurement (LAM) software (Muchchadia and Thaker 2017) which showed leaf area between 0.76 to 8.86 $cm^2$. The fresh weight 34-182.5 mg $leaf^{-1}$, dry weight 20.2 to 173 mg $leaf^{-1,}$ and water content of 11.3 to 62 mg $leaf^{-1}$, was observed (Fig. 5). The leaf area was plotted against the fresh weight, dry weight, and water content. The figures showed a linear relationship between leaf area against all gravimetric parameters. Data on leaf area and gravimetric parameters showed a statistically significant linear relationship $R^2$ = 0.9068 (fresh weight), $R^2$ = 0.8729 (water content), and $R^2$ = 0.8640 (dry weight) (Table 1, Fig. 1). The correlation coefficient was calculated (Fig. 2) for leaf area, fresh and dry weight water content, and chlorophyll content. The leaf area of *B. aegyptiaca* showed a highly significant correlation with fresh weight ($r$ = 0.994, P <0.001), dry weight ($r$ = 0.995, P < 0.001), and water content ($r$ = 0.993, P < 0.001). Data of gravimetric analysis for leaf area suggested that water content is more significant than other parameters (Table 1) and thus it can be used as a linear model for *B. aegyptiaca.*

**Table 1:** Results of Regression analysis of *B. aegyptiaca* and *G. montana*

| Plant name | Parameter | Regression equation | $R^2$ |
|---|---|---|---|
| | Dry weight | $y = 9.786x - 2.464$ | 0.864 |
| *B. aegyptiaca* | Water content | $y = 17.32x - 6.652$ | 0.872 |
| | Fresh weight | $y = 27.108x - 9.1177$ | 0.906 |
| | Dry weight | $y = 11.79x - 5.490$ | 0.940 |
| *G. montana* | Water content | $y = 23.32x - 12.92$ | 0.895 |
| | Fresh weight | $y = 34.772x - 18.237$ | 0.902 |

Similarly, for *G. montana,* 200 leaves were tested for the development of the linear model. The leaf area of *G. montana* was observed in the range between 1.2 to 14.63 $cm^2$ $leaf^{-1}$. The fresh and dry weights observed in the range of 54-349 mg $leaf^{-1}$ and 15 to115 mg $leaf^{-1}$, respectively. The water content was observed in the range of 29-234 mg $leaf^{-1}$ (Fig. 6). The leaf area was plotted against the fresh weight, dry weight, and water content. The figures showed the linear relationship between

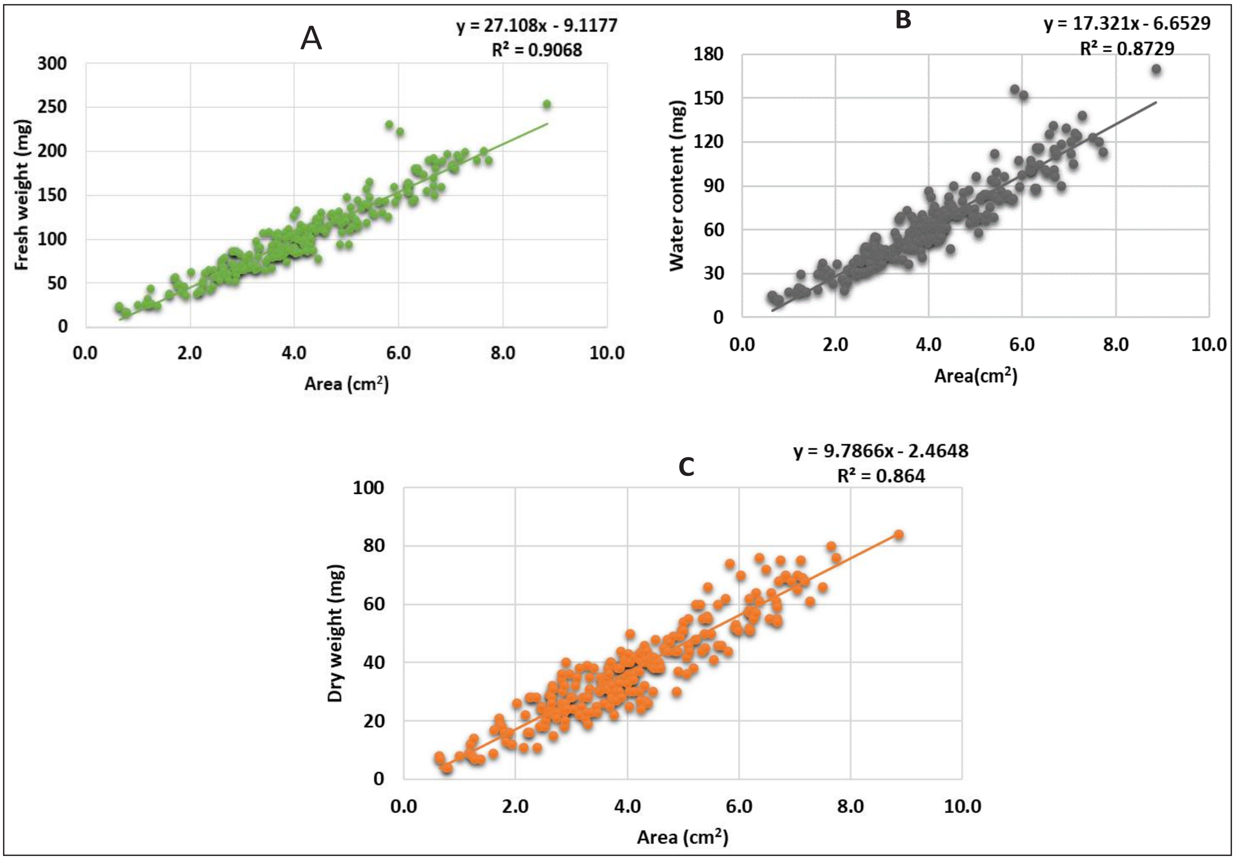


**Fig. 1:** Leaf area linear model for *B. aegyptiaca* using gravimetric parameters **(A)** Fresh weight **(B)** water content and **(C)** dry weight of each leaf

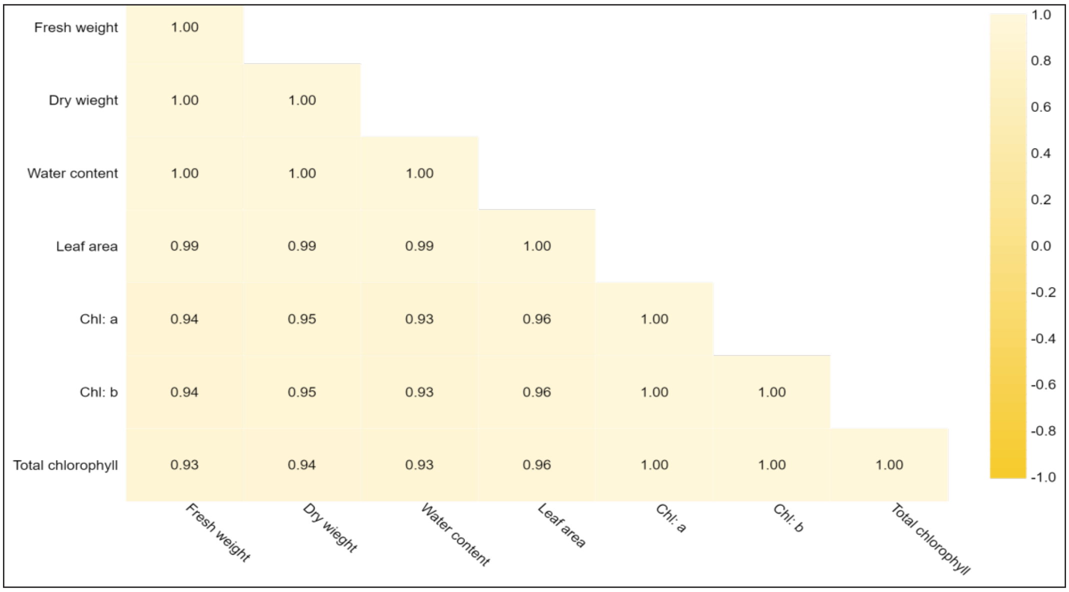


**Fig. 2:** Correlation coefficient 'r' between gravimetric parameters and chlorophyll content of *B. aegyptiaca*

leaf area against all gravimetric parameters. Data on leaf area and gravimetric parameters showed statistically significant linear relationship $R^2 = 0.9033$ (fresh weight), $R^2 = 0.8484$ (water content) and $R^2 = 0.9283$ (dry weight) (Fig. 3, Table 1). Leaf area of *G. montana* showed a highly significant correlation with fresh weight ($r = 0.993$, $P<0.001$), dry weight ($r = 0.999$, $P<0.001$), and water content ($r = 0.998$, $P < 0.001$) (Fig. 4).

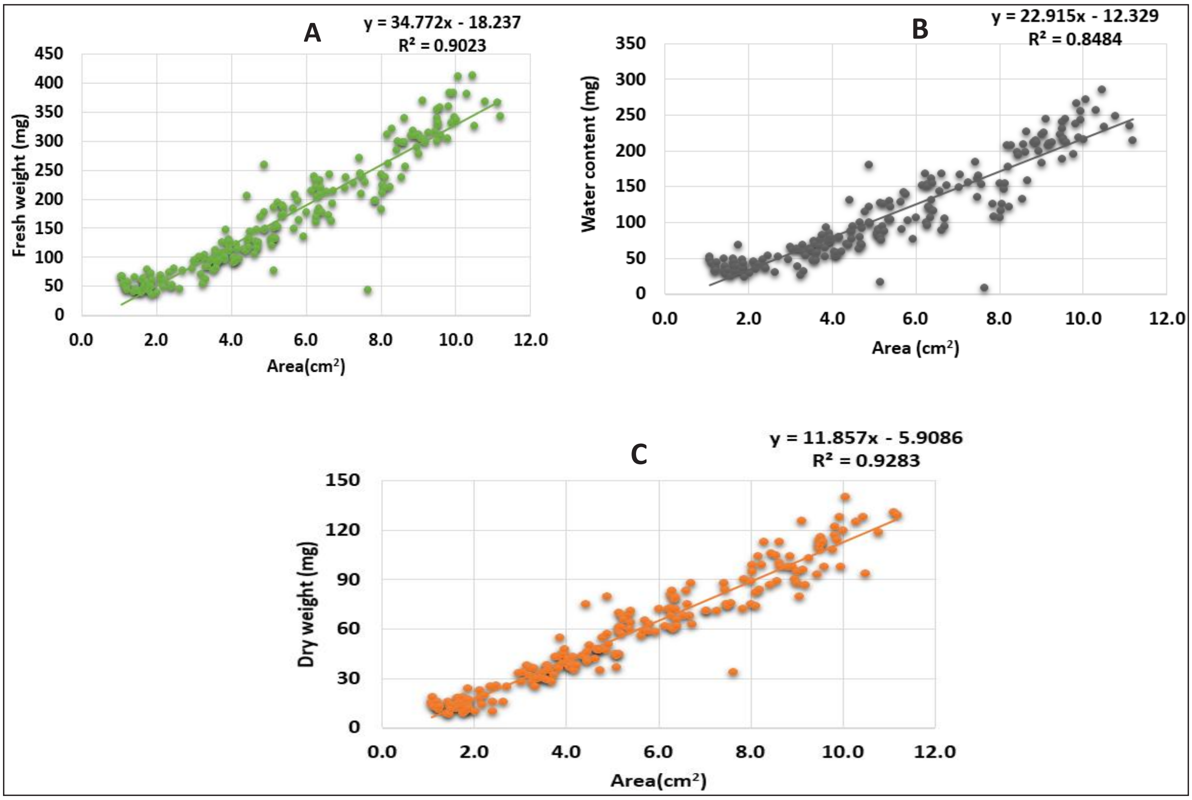


**Fig. 3:** Leaf area linear model for *G. montana* using gravimetric parameter **(A)** Fresh weight **(B)** water content and **(C)** dry weight of each leaf

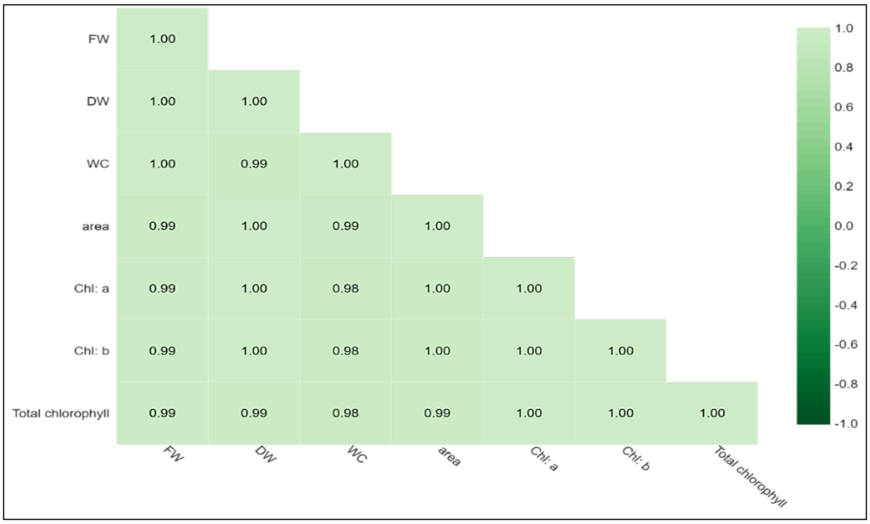


**Fig. 4:** Correlation coefficient 'r' between gravimetric parameters and chlorophyll content of *G. montana*

Similarly, the data of chlorophyll content of *B. aegyptiaca*, and *G. montana* was also increased with the leaf size. The total chlorophyll was gradually increased with an increase in leaf size and deceased at the latter age of leaf due to the senescence (Figs. 7A, and 7B). Liu *et al.* (2019) reported that leaf growth and chlorophyll content relationship maintain constant throughout biomass yield. Chlorophyll pigment shows the good antioxidant property of neutralizing free radicals in the body (Lanfer-Marquez *et al.* 2005). This natural antioxidant was used against cancer atherosclerosis,arthritis,and cardiovascular and neurodegenerative diseases (Sarke *et al.* 2020). We have used chlorophyll content considering the fact that chlorophyll content also increases with increasing in the size of the leaf.

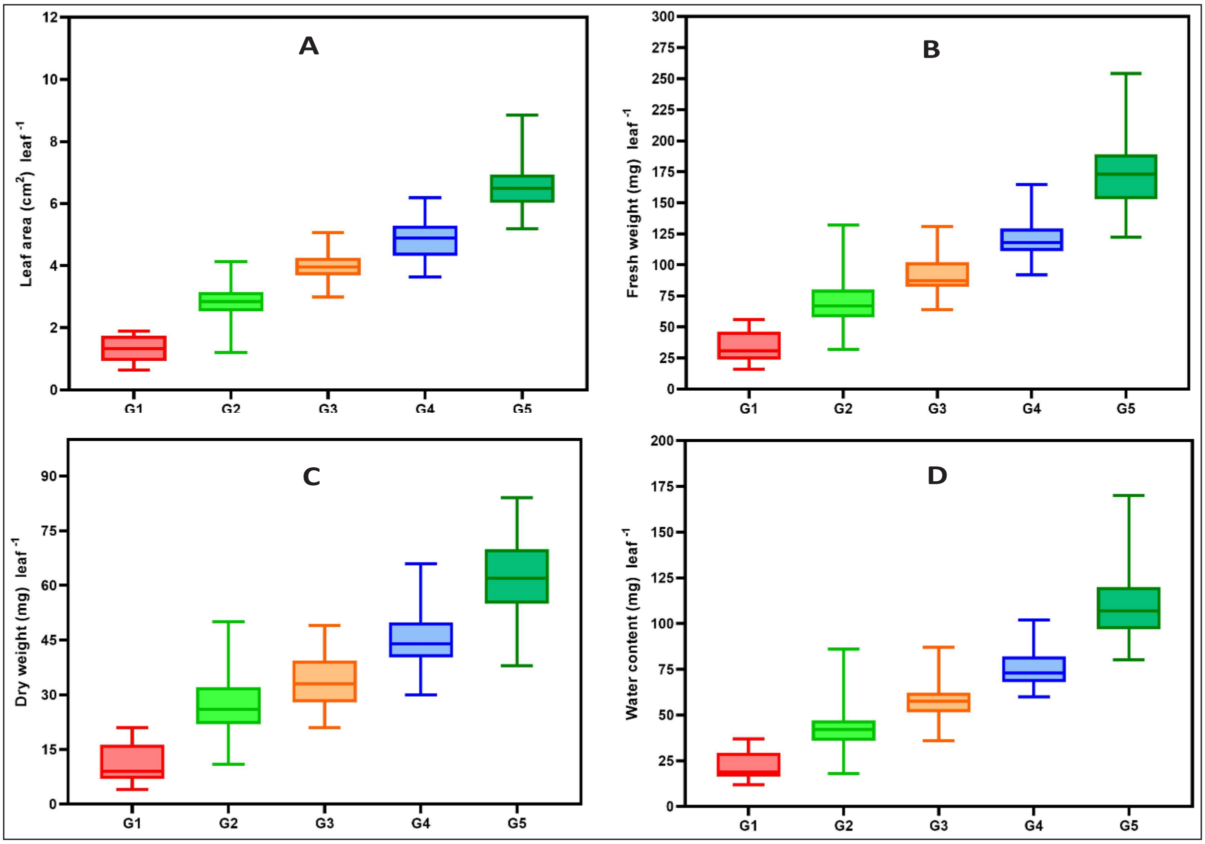


**Fig. 5:** Range of selected leaf **(A)** leaf area **(B)** Fresh weight **(C)** Dry weight **(D)** water content of *B. aegyptiaca*

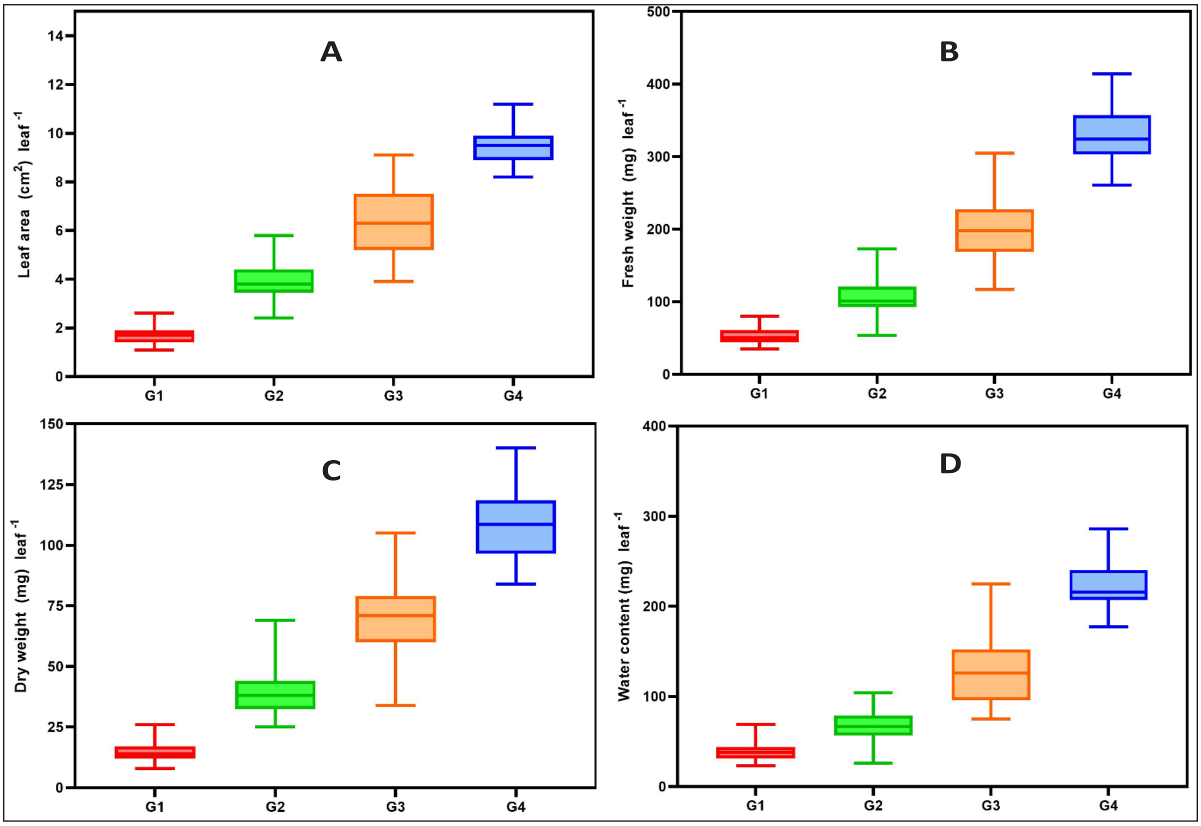


**Fig. 6:** Range of selected leaf **(A)** leaf area **(B)** Fresh weight **(C)** Dry weight **(D)** water content of *G. montana*

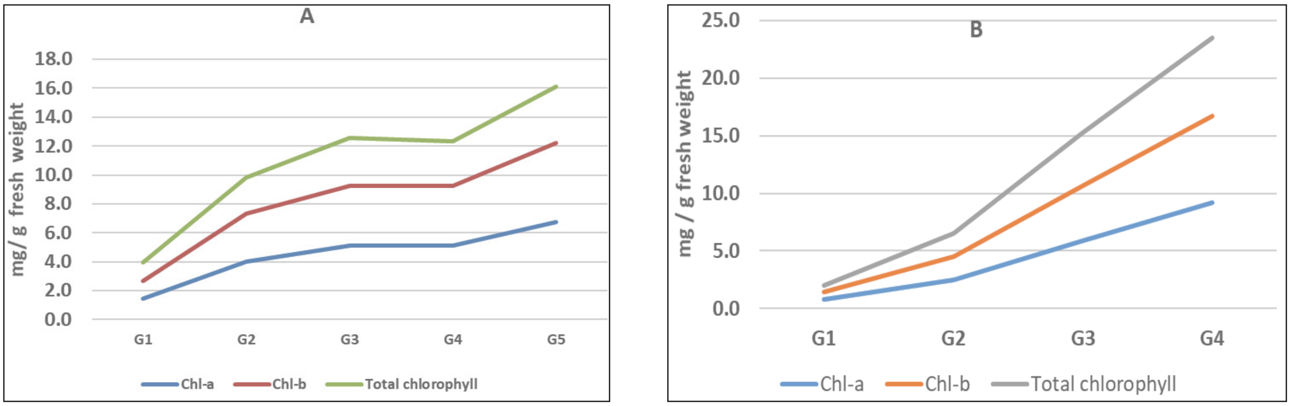


**Fig. 7:** Changes in chlorophyll content with leaf a size in **(A)** *B. aegyptiaca* **(B)** *G. montana*

Thus these results on the leaf size and chlorophyll content may help in designing herbal formulations for effective drug preparation.

Many authors used dry weight as the representative of leaf biomass when studying the relationship between leaf biomass and area (Wilson *et al.* 2001; Lee and Heuvelink, 2003; Young *et al.* 2007; Lin *et al.* 2020). In many studies, the dry weight is reported as a considerable trait in 93 temperate woody species (Li *et al.* 2008), bamboo species (Huang *et al.* 2019), and oil palm (Awal *et al.* 2004). Leaf dry mass or its equal leaf area is an important trait that is closely associated with plant growth rates, photosynthetic rate, reproductive strategies, and life span (Poorter *et al.* 2009; Falster *et al.* 2018). In this work, all the data of dry weight against the leaf area was highly significant at $P < 0.001$, therefor dry weight may be considered for the linear model development in *B. aegyptiaca*, and *G. montana* plants.

Regression and correlation analysis demonstrated that there was a significant relationship between water content and leaf area. Water content is also an important parameter to study the role of water in the expansion growth of the plant (Patel and Thaker 2007; Monpara *et al.* 2019). A number of the studies conducted in our lab and elsewhere observed that dry weight accumulation and water content are closely related to the expansion growth of the plant (Egli, 1990; Chudasama and Thaker 2007; Bhatt and Thaker 2008). It is assumed that an increase in the size of the leaf may also increase the water content up to a certain size leaf. A significant correlation was observed between water content and dry weight with leaf area in *B. aegyptiaca* and *G. montana* Similar results were also reported for *Adhatoda vasica* (Thaker *et al.* 2016), *Vitex negundo*, and *Helicteres isora* (Kumbhani *et al.* 2017).

Regression and correlation analysis revealed that leaf area with water content, dry and fresh weight was statistically significant ($P< 0.001$) in all tested plants. Leaf area is an important variable in studies related to their growth and development because leaves are synthesized with the carbohydrates and transported to other organs of the plant (Caliskan *et al.* 2010). It is a major parameter in plant modeling studies, to develop physiological models in plant breeding and horticulture crops (Gao *et al.* 2012; Khan *et al.* 2016).

In the model validation, very close relationships were observed leaf area with dry weight and water content using the linear model in all tested plants. Therefore, it is suggested that the leaf area with its specific leaf size will be used as a guideline for the harvest of *B. aegyptiaca* and *G. montana* very important medicinal plants for the preparation of effective herbal formulation. However, characterization of chemical composition with leaf size is needed for further research.

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